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NEWS 4	Feb 16	TOXLINE no longer being updated
NEWS 5	Apr 23	Search Derwent WPINDEX by chemical structure
NEWS 6	Apr 23	PRE-1967 REFERENCES NOW SEARCHABLE IN CAPLUS AND CA
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=> s alpha lipoic acid

L1 1889 ALPHA LIPOIC ACID

=> s protein or amino acid or glutamine or leucine or isoleucine or valine or arginine or alanine or whey protein or WPI 97 or WPC 80

2 FILES SEARCHED...
4 FILES SEARCHED...
L2 6149934 PROTEIN OR AMINO ACID OR GLUTAMINE OR LEUCINE OR ISOLEUCINE OR VALINE OR ARGININE OR ALANINE OR WHEY PROTEIN OR WPI 97 OR WPC 80

=> s carbohydrate or creatine or ginseng or cysteine or phenylalanine or ascorbic acid or inositol or pinitol or tocopherol or sadium or patssium or phosphorus

4 FILES SEARCHED...
L3 1595628 CARBOHYDRATE OR CREATINE OR GINSENG OR CYSTEINE OR PHENYLALANINE
OR ASCORBIC ACID OR INOSITOL OR PINITOL OR TOCOPHEROL OR SADIUM
OR PATSSIUM OR PHOSPHORUS

=> s l1 and l2 and l3

L4 207 L1 AND L2 AND L3

=> s l4 and muscle mass

L5 5 L4 AND MUSCLE MASS

=> s l5 and py<1999

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4 FILES SEARCHED...
L6 0 L5 AND PY<1999

=> s l4 and py<1999

2 FILES SEARCHED...
4 FILES SEARCHED...
L7 82 L4 AND PY<1999

=> d l7 1-10 kwic ab bib

L7 ANSWER 1 OF 82 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
SO Free Radical Biology and Medicine, (1998) 26/1-2 (174-183).
Refs: 55
ISSN: 0891-5849 CODEN: FRBMEH
AB . . . signalling pathways leading to gene expression has not been clearly established. In the present study, the effects of the antioxidants
.alpha.-lipoic acid, N-acetyl-L-
cysteine (NAC) and the flavonoid extract silymarin were investigated for their ability to modulate- the activation of the transcription factors nuclear factor kappa B (NF-.kappa.B) and activator **protein**-1 (AP-1) in HaCaT keratinocytes after exposure to a solar UV simulator. The activation of NF-.kappa.B and AP-1 showed a similar. . . were evaluated 3 h after exposure. While a high concentration of NAC could achieve a complete inhibition, low concentrations of **.alpha .-lipoic acid** and silymarin were shown to

significantly inhibit NF-.kappa.B activation. In contrast, AP-1 activation
was only partially inhibited by NAC, and not at all by **.alpha.-lipoic acid** or silymarin. These results indicate that antioxidants such as **.alpha.-lipoic acid** and silymarin can efficiently modulate the cellular response to UVR through their selective action on NF-.kappa.B activation.

CT Medical Descriptors:
*keratinocyte
*ultraviolet radiation
gene expression
skin cancer
skin disease
cytotoxicity
electrophoretic mobility
human
controlled study
human cell
article
priority journal
*antioxidant
***immunoglobulin enhancer binding protein: EC, endogenous compound**
*transcription factor ap 1: EC, endogenous compound
*thioctic acid
*acetylcysteine
*silymarin
lactate dehydrogenase: EC, endogenous compound

AB Exposure of the human skin to ultraviolet radiation (UVR) leads to depletion of cutaneous antioxidants, regulation of gene expression and ultimately to the development of skin diseases. Although exogenous supplementation of antioxidants prevents UVR-induced photooxidative damage, their effects on components of cell signalling pathways leading to
gene expression has not been clearly established. In the present study, the effects of the antioxidants **.alpha.-lipoic acid**, N-acetyl-L-**cysteine** (NAC) and the flavonoid extract silymarin were investigated for their ability to modulate- the activation of the transcription factors nuclear factor kappa B (NF-.kappa.B) and activator **protein**-1 (AP-1) in HaCaT keratinocytes after exposure to a solar UV simulator. The activation of NF-.kappa.B and AP-1 showed a similar temporal pattern: activation was detected 2 h after UV exposure and maintained for up to 8 h. To determine the capacity of activated NF-.kappa.B to stimulate transcription, NF-.kappa.B-dependent gene expression was measured using a reporter gene assay. The effects of the antioxidants on NF-.kappa.B and AP-1 activation were evaluated 3 h after exposure. While a high concentration of NAC could
achieve a complete inhibition, low concentrations of **.alpha.-lipoic acid** and silymarin were shown to significantly inhibit NF-.kappa.B activation. In contrast, AP-1 activation was only partially inhibited by NAC, and not at all by **.alpha.-lipoic acid** or silymarin. These results indicate that antioxidants such as **.alpha.-lipoic acid** and silymarin can efficiently modulate the cellular response to UVR through their selective action on NF-.kappa.B activation.

AN 1999000785 EMBASE

TI Antioxidants modulate acute solar ultraviolet radiation-induced NF-.kappa.-B activation in a human keratinocyte cell line.

AU Saliou C.; Kitazawa M.; McLaughlin L.; Yang J.-P.; Lodge J.K.; Tetsuka T.;
Iwasaki K.; Cillard J.; Okamoto T.; Packer L.

CS Dr. L. Packer, Membrane Bioenergetics Group, 251 Life Sciences Addition, Department of Molecular/Cell Biology, Berkeley, CA 94720-3200, United States. packer@socrates.berkeley.edu

SO Free Radical Biology and Medicine, (1998) 26/1-2 (174-183).
Refs: 55

ISSN: 0891-5849 CODEN: FRBMEH
PUI S 0891-5849(98)00212-3
CY United States
DT Journal; Article
FS 013 Dermatology and Venereology
029 Clinical Biochemistry
LA English
SL English

L7 ANSWER 2 OF 82 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
SO Diabetes und Stoffwechsel, (20 Nov 1998) 7/6 (251-266).
Refs: 134
ISSN: 0942-0037 CODEN: DISTF5
AB . . . vascular factors: 1. Increased flux through the polyol pathway that leads to accumulation of sorbitol and Fructose, depletion of myo-**inositol**, reduction in Na+-K+-ATPase activity and alterations in the expression of several isoenzymes of **protein** kinase C (PKC); 2. Disturbances in n-6 essential fatty acid and prostaglandin metabolism which result in alterations of nerve membrane. . . and hypoxia as well as generation of reactive oxygen species (oxidative stress) and the so called oil, administration of antioxidants (.**alpha**.-**lipoic acid**) to reduce the enhanced formation of reactive oxygen species that induce increased oxidative stress, improvement in endoneurial blood flow and. . .
CT Medical Descriptors:
*diabetic neuropathy: CO, complication
*diabetic neuropathy: ET, etiology
*diabetic neuropathy: PC, prevention
diabetes mellitus
disease classification
neurological complication: CO, complication
neurological complication: ET, etiology
neurological complication: PC, prevention
pathogenesis
protein expression
prostaglandin metabolism
oxidative stress
hyperglycemia
human
nonhuman
rat
major clinical study
clinical trial
multicenter study
animal model
controlled study
article
*nerve growth factor
*antioxidant
*thioctic acid
*dipeptidyl carboxypeptidase inhibitor
*prostaglandin derivative
adenosine triphosphatase (potassium sodium): EC, endogenous compound
protein kinase c: EC, endogenous compound
neurotrophin 3: EC, endogenous compound
somatomedin c: EC, endogenous compound
polyol
sorbitol
inositol
gamma linolenic acid
primrose oil
aldose reductase inhibitor
nitric oxide synthase inhibitor
n(g) nitroarginine
prostaglandin synthase inhibitor
flurbiprofen

glucocorticoid
immunosuppressive agent
immunoglobulin
vasodilator agent
trandolapril
carvedilol
lisinopril
prostaglandin e1 derivative
17,20 dimethyl 7 thiaprostaglandin e1 methyl. . .

RN (nerve growth factor) 9061-61-4; (thioctic acid) 1077-29-8, 1200-22-2, 2319-84-8, 62-46-4; (**protein** kinase c) 141436-78-4; (somatomedin c) 67763-96-6; (sorbitol) 26566-34-7, 50-70-4, 53469-19-5; (**inositol**) 55608-27-0, 6917-35-7, 87-89-8; (gamma linolenic acid) 1686-12-0; (primrose oil) 65546-85-2; (n(g) nitroarginine) 2149-70-4; (flurbiprofen) 5104-49-4; (immunoglobulin) 9007-83-4; (trandolapril) 87679-37-6; (carvedilol). . .

AB Recent experimental studies suggest a multifactorial pathogenesis of diabetic neuropathy. Most data have been generated in the diabetic rat model, on the basis of which two approaches have been chosen to contribute to the clarification of the pathogenesis of diabetic neuropathy. Firstly, it has been attempted to characterize the pathophysiological, pathobiochemical, and structural abnormalities that result in experimental diabetic neuropathy. Secondly, specific therapeutic interventions have been employed to prevent the development of these alterations, to halt their progression, or to induce their regression despite concomitant hyperglycaemia. At present, the following six pathogenetic mechanisms are being discussed which, however, in contrast to previous years, are no longer regarded as separate hypotheses but in the first place as a complex interplay with multiple interactions between metabolic and vascular factors: 1. Increased flux through the polyol pathway that leads to accumulation of sorbitol and Fructose, depletion of myo- **inositol**, reduction in Na+-K+-ATPase activity and alterations in the expression of several isoenzymes of **protein** kinase C (PKC); 2. Disturbances in n-6 essential fatty acid and prostaglandin metabolism which result in alterations of nerve membrane structure and microvascular and haemorrheologic abnormalities; 3. Endoneurial microvascular deficits with subsequent ischaemia and hypoxia as well as generation of reactive oxygen species (oxidative stress) and the so called oil, administration of antioxidants (.**alpha**.- **lipoic acid**) to reduce the enhanced formation of reactive oxygen species that induce increased oxidative stress, improvement in endoneurial blood flow and resulting hypoxia by vasodilating agents such as ACE inhibitors and prostaglandin analogues, neurotrophic support by administration of NGF, inhibition of non-enzymatic glycation and formation of AGEs by aminoguanidine and immunosuppressive treatment. Since in the foreseeable future (near-)normoglycaemia will not be achievable in the majority of diabetic patients, the advantage of the aforementioned treatment approaches is that they may exert their effects despite prevailing hyperglycaemia. In future, combinations of certain drugs that produce synergistic effects could be used as therapeutic options.

AN 1998410691 EMBASE

TI [Pathogenesis of diabetic neuropathy].
PATHOGENESE DER DIABETISCHEN NEUROPATHIE.

AU Ziegler D.
CS Dr. D. Ziegler, Diabetes-Forschungsinstitut, Heinrich-Heine-Universitat, Klinische Abteilung, Auf'm Hennekamp 65, 40225 Dusseldorf, Germany

SO Diabetes und Stoffwechsel, (20 Nov 1998) 7/6 (251-266).
Refs: 134
ISSN: 0942-0037 CODEN: DISTF5
CY Germany
DT Journal; Article
FS 006 Internal Medicine

008 Neurology and Neurosurgery
037 Drug Literature Index
LA German
SL English; German

L7 ANSWER 3 OF 82 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
SO Biochemical Pharmacology, (1 Jun 1998) 55/11 (1747-1758).
Refs: 134
ISSN: 0006-2952 CODEN: BCPCA6
AB . . . a fundamental regulatory mechanism in cell biology. Electron flow through side chain functional CH2-SH groups of conserved cysteinyl residues in **proteins** account for their redox-sensing properties. Because in most intracellular **proteins** thiol groups are strongly 'buffered' against oxidation by the highly reduced environment inside the cell, only accessible **protein** thiol groups with high thiol-disulfide oxidation potentials are likely to be redox sensitive. The list of redox- sensitive signal transduction. . . are of central importance in redox signaling. Among the thiol agents tested for their efficacy to modulate cellular redox status, N-acetyl-L-**cysteine** (NAC) and .**alpha**.-**lipoic acid** hold promise for clinical use. A unique advantage of lipoate is that it is able to utilize cellular reducing equivalents,. . . regenerate its reductive vicinal dithiol form. Because lipoate can be readily recycled in the cell, it has an advantage over N-acetyl-L-**cysteine** on a concentration:effect basis. Our current knowledge of redox regulated signal transduction has led to the unfolding of the remarkable. . .
CT Medical Descriptors:
*oxidation reduction reaction
*signal transduction
*gene expression
electron transport
oxidative stress
protein dna binding
structure activity relation
enzyme activity
cancer: DR, drug resistance
oncogene c jun
review
priority journal
*thiol group
*antioxidant: PD, pharmacology
*acetylcysteine: PD, pharmacology
*thioctic acid: PD, pharmacology
immunoglobulin enhancer binding protein: EC, endogenous compound
oxidoreductase: EC, endogenous compound
transforming growth factor beta1: EC, endogenous compound
zinc finger protein: EC, endogenous compound
protein tyrosine phosphatase: EC, endogenous compound
protein kinase: EC, endogenous compound
ryanodine receptor: EC, endogenous compound
thioredoxin: EC, endogenous compound
protein c fos: EC, endogenous compound
glutathione: EC, endogenous compound
doxorubicin: PD, pharmacology
RN (acetylcysteine) 616-91-1; (thioctic acid) 1077-29-8, 1200-22-2, 2319-84-8, 62-46-4; (oxidoreductase) 9035-73-8, 9035-82-9, 9037-80-3, 9055-15-6; (**protein** tyrosine phosphatase) 79747-53-8, 97162-86-2; (**protein** kinase) 9026-43-1; (thioredoxin) 52500-60-4; (glutathione) 70-18-8; (doxorubicin) 23214-92-8, 25316-40-9
AB Oxidation-reduction (redox) based regulation of signal transduction and gene expression is emerging as a fundamental regulatory mechanism in cell biology. Electron flow through side chain functional CH2-SH groups of conserved cysteinyl residues in **proteins** account for their

redox-sensing properties. Because in most intracellular **proteins** thiol groups are strongly 'buffered' against oxidation by the highly reduced environment inside the cell, only accessible **protein** thiol groups with high thiol-disulfide oxidation potentials are likely to be redox sensitive. The list of redox- sensitive signal transduction pathways is steadily growing, and current information suggests that manipulation of the cell redox state may prove to be an important strategy for the management of AIDS and some forms of cancer. The endogenous thioredoxin and glutathione systems are of central importance in redox signaling. Among the thiol agents tested for their efficacy to modulate cellular redox status, N-acetyl-L-**cysteine** (NAC) and . **alpha.-lipoic acid** hold promise for clinical use. A unique advantage of lipoate is that it is able to utilize cellular reducing equivalents, and thus it harnesses the metabolic power of the cell to continuously regenerate its reductive vicinal dithiol form. Because lipoate can be readily recycled in the cell, it has an advantage over N-acetyl-L-**cysteine** on a concentration:effect basis. Our current knowledge of redox regulated signal transduction has led to the unfolding of the remarkable therapeutic potential of cellular thiol modulating agents.

AN 1998221925 EMBASE

TI Redox signaling and the emerging therapeutic potential of thiol antioxidants.

AU Sen C.K.

CS Dr. C.K. Sen, 251 Life Sciences Addition, Dept. of Molecular and Cell Biology, University of California, Berkeley, CA 94720-3200, United States.
cksen@socrates.berkeley.edu

SO Biochemical Pharmacology, (1 Jun 1998) 55/11 (1747-1758).
Refs: 134
ISSN: 0006-2952 CODEN: BCPCA6

PUI S 0006-2952(97)00672-2

CY United States

DT Journal; General Review

FS 029 Clinical Biochemistry
030 Pharmacology
037 Drug Literature Index

LA English

SL English

L7 ANSWER 4 OF 82 EMBASE

SO Biochemistry and Molecular Biology International, (1997) 42/6 (1189-1197).
Refs: 32
ISSN: 1039-9712 CODEN: BMBIES

AB The effect of several antioxidants and **cysteine**-elevating precursor drugs (prodrugs) was tested on lens damage occurring after in vitro exposure to low levels of 60Co-y-irradiation, to simulate in vitro the exposure to radiation in vivo of (1) astronauts (2) jet crews (3) military radiation accident personnel. **Tocopherol** (100 .mu.M), **ascorbic acid** (1 mM), R-.**alpha.-lipoic acid** (1 mM), and taurine (0.5 mM) protected against radiation-associated **protein** leakage. MTCA and ribocysteine protected lenses against opacification, LDH and **protein** leakage, indicating that antioxidants and prodrugs of **cysteine** appear to offer protection against lens damage caused by low level radiation.

CT Medical Descriptors:
*cataract: ET, etiology
space
aging
gamma irradiation
simulation
cosmonaut
radiation injury
nonhuman

rat
controlled study
animal tissue
article
*antioxidant
cobalt 60
tocopherol
ascorbic acid
thioctic acid
taurine

RN (cobalt 60) 10198-40-0; (**tocopherol**) 1406-66-2; (**ascorbic acid**) 134-03-2, 15421-15-5, 50-81-7; (thioctic acid) 1077-29-8, 1200-22-2, 2319-84-8, 62-46-4; (taurine) 107-35-7

AB The effect of several antioxidants and **cysteine**-elevating precursor drugs (prodrugs) was tested on lens damage occurring after in vitro exposure to low levels of 60Co-y-irradiation, to simulate in vitro the exposure to radiation in vivo of (1) astronauts (2) jet crews (3) military radiation accident personnel. **Tocopherol** (100 .mu.M), **ascorbic acid** (1 mM), R-.**alpha.**-**lipoic acid** (1 mM), and taurine (0.5 mM) protected against radiation-associated **protein** leakage. MTCA and ribocysteine protected lenses against opacification, LDH and **protein** leakage, indicating that antioxidants and prodrugs of **cysteine** appear to offer protection against lens damage caused by low level radiation.

AN 1998215120 EMBASE

TI Antioxidants and cataract: (cataract induction in space environment and application to terrestrial aging cataract).

AU Bantseev V.; Bhardwaj R.; Rathbun W.; Nagasawa H.; Trevithick J.R.

CS V. Bantseev, Department of Biochemistry, University of Western Ontario, London, Ont. N6A 5C1, Canada

SO Biochemistry and Molecular Biology International, (1997) 42/6 (1189-1197).
Refs: 32
ISSN: 1039-9712 CODEN: BMBIES

CY Australia

DT Journal; Article

FS 005 General Pathology and Pathological Anatomy
012 Ophthalmology

LA English

SL English

L7 ANSWER 5 OF 82 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.

TI .**alpha.**-**Lipoic acid**: A metabolic antioxidant which regulates NF-.kappa.B signal transduction and protects against oxidative injury.

SO Drug Metabolism Reviews, (1998) 30/2 (245-275).
Refs: 113
ISSN: 0360-2532 CODEN: DMTRAR

AB . . . modulating transcription factor activity, especially that of NF-.kappa.B (Fig. 12). These mechanisms may account for the sometimes dramatic effects of .**alpha.**-**lipoic acid** in oxidative stress conditions (e.g., brain ischemia-reperfusion), and point the way toward its therapeutic use.

CT Medical Descriptors:
*oxidative stress
antioxidant activity
signal transduction
transcription regulation
cataract: ET, etiology
alpha tocopherol deficiency: ET, etiology
reperfusion injury: ET, etiology
brain ischemia: ET, etiology
neurotoxicity: ET, etiology
virus inhibition
human immunodeficiency virus
oxidation reduction state

calcium cell level
glutathione metabolism
human
nonhuman
conference paper
*thioctic acid: EC, endogenous compound
*antioxidant: EC, endogenous compound
***immunoglobulin enhancer binding protein: EC, endogenous compound**
dihydrolipoate: EC, endogenous compound
alpha tocopherol: EC, endogenous compound
excitotoxin: EC, endogenous compound
amino acid receptor affecting agent: EC, endogenous compound
calcium ion: EC, endogenous compound
glutathione: EC, endogenous compound

RN (thioctic acid) 1077-29-8, 1200-22-2, 2319-84-8, 62-46-4; (dihydrolipoate) 462-20-4; (alpha **tocopherol**) 1406-18-4, 1406-70-8, 52225-20-4, 58-95-7, 59-02-9; (calcium ion) 14127-61-8; (glutathione) 70-18-8

AB Although the metabolic role of .alpha.-lipid acid has been known for over 40 years, it is only recently that its effects when supplied exogenously have become known. Exogenous .alpha.-lipoic is reduced intracellularly by at least two and possibly three enzymes, and through the actions of its reduced form, it influences a number of cell process. These include direct radical scavenging, recycling of other antioxidants, accelerating GSH synthesis, and modulating transcription factor activity, especially that of NF-.kappa.B (Fig. 12). These mechanisms may account for the sometimes dramatic effects of **.alpha.-lipoic acid** in oxidative stress conditions (e.g., brain ischemia-reperfusion), and point the way toward its therapeutic use.

AN 1998192814 EMBASE

TI **.alpha.-Lipoic acid**: A metabolic antioxidant which regulates NF-.kappa.B signal transduction and protects against oxidative injury.

AU Packer L.

CS L. Packer, Dept. of Molecular and Cell Biology, University of California, 251 Life Sciences Addition, Berkeley, CA 94720-3200, United States

SO Drug Metabolism Reviews, (1998) 30/2 (245-275).
Refs: 113
ISSN: 0360-2532 CODEN: DMTRAR

CY United States

DT Journal; Conference Article

FS 005 General Pathology and Pathological Anatomy
029 Clinical Biochemistry

LA English

SL English

L7 ANSWER 6 OF 82 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.

TI **.alpha.-lipoic acid** in liver metabolism and disease.

SO Free Radical Biology and Medicine, (1998) 24/6 (1023-1039).
Refs: 141
ISSN: 0891-5849 CODEN: FRBMEH

AB R-**.alpha.-Lipoic acid** is found naturally occurring as a prosthetic group in .alpha.-keto acid dehydrogenase complexes of the mitochondria, and as such plays a fundamental role in metabolism. Although this has been known for decades, only recently has free supplemented **.alpha.-lipoic acid** been found to affect cellular metabolic processes in vitro, as it has the ability to alter the redox status of. . . it appears that this compound has important therapeutic potential in conditions where oxidative stress is involved. Early case studies with **.alpha.- lipoic acid** were performed with little knowledge of the action of **.alpha.-lipoic acid** at a cellular level, but with the rationale that because the naturally occurring **protein**

bound form of **.alpha.-lipoic acid** has a pivotal role in metabolism, that supplementation may have some beneficial effect. Such studies sought to evaluate the effect of supplemented **.alpha.-lipoic acid**, using low doses, on lipid or **carbohydrate** metabolism, but little or no effect was observed. A common response in these trials was an increase in glucose uptake,. . . lactate were also observed, suggesting that an inhibitory effect on the pyruvate dehydrogenase complex was occurring. During the same period, **.alpha.-lipoic acid** was also used as a therapeutic agent in a number of conditions relating to liver disease, including alcohol-induced damage, mushroom poisoning, metal intoxification, and CCl4 poisoning. **.alpha.-Lipoic acid** supplementation was successful in the treatment for these conditions in many cases. Experimental studies and clinical trials in the last 5 years using high doses of **.alpha.-lipoic acid** (600 mg in humans) have provided new and consistent evidence for the therapeutic role of antioxidant **.alpha.-lipoic acid** in the treatment of insulin resistance and diabetic polyneuropathy. This new insight should encourage clinicians to use . **alpha.-lipoic acid** in diseases affecting liver in which oxidative stress is involved.

CT Medical Descriptors:
*liver . . . drug therapy
alcohol liver disease: ET, etiology
mushroom poisoning: DT, drug therapy
mushroom poisoning: ET, etiology
insulin resistance
diabetic neuropathy: CO, complication
diabetic neuropathy: DT, drug therapy
carbohydrate metabolism
lipid metabolism
biliary cirrhosis: DT, drug therapy
human
nonhuman
clinical trial
review
priority journal
*thioctic acid: CT, clinical trial
*thioctic acid: AD, drug administration
*thioctic acid: DO, drug. . .

AB R-**.alpha.-Lipoic acid** is found naturally occurring as a prosthetic group in .alpha.-keto acid dehydrogenase complexes of the mitochondria, and as such plays a fundamental role in metabolism. Although this has been known for decades, only recently has free supplemented **.alpha.-lipoic acid** been found to affect cellular metabolic processes in vitro, as it has the ability to alter the redox status of cells and interact with thiols and other antioxidants. Therefore, it appears that this compound has important therapeutic potential in conditions where oxidative stress is involved. Early case studies with **.alpha.- lipoic acid** were performed with little knowledge of the action of **.alpha.-lipoic acid** at a cellular level, but with the rationale that because the naturally occurring **protein** bound form of . **alpha.-lipoic acid** has a pivotal role in metabolism, that supplementation may have some beneficial effect. Such studies sought to evaluate the effect of supplemented **.alpha.-lipoic acid**, using low doses, on lipid or **carbohydrate** metabolism, but little or no effect was observed. A common response in these trials was an increase in glucose uptake, but increased plasma levels of pyruvate and lactate were also observed, suggesting that an inhibitory effect on the pyruvate dehydrogenase complex was occurring. During the same period, **.alpha.-lipoic**

mushroom poisoning, metal intoxification, and CCl4 poisoning. . **alpha.-Lipoic acid** supplementation was successful in the treatment for these conditions in many cases. Experimental studies and clinical trials in the last 5 years using high doses of **.alpha.-lipoic acid** (600 mg in humans) have provided new and consistent evidence for the therapeutic role of antioxidant **.alpha.-lipoic acid** in the treatment of insulin resistance and diabetic polyneuropathy. This new insight should encourage clinicians to use **.alpha.-lipoic acid** in diseases affecting liver in which oxidative stress is involved.

AN 1998136441 EMBASE
TI **.alpha.-lipoic acid** in liver metabolism and disease.
AU Bustamante J.; Lodge J.K.; Marcocci L.; Tritschler H.J.; Packer L.; Rihn B.H.
CS L. Packer, Membranes Bioenergetics Group, Dept. of Molecular and Cell Biology, University of California, Berkeley, CA 94720-3200, United States
SO Free Radical Biology and Medicine, (1998) 24/6 (1023-1039).
Refs: 141
ISSN: 0891-5849 CODEN: FRBMEH
PUI S 0891-5849(97)00371-7
CY United States
DT Journal; General Review
FS 005 General Pathology and Pathological Anatomy
029 Clinical Biochemistry
030 Pharmacology
037 Drug Literature Index
048 Gastroenterology
LA English
SL English

L7 ANSWER 7 OF 82 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
TI Advanced glycation end product-induced activation of NF-.kappa.B is suppressed by **.alpha.-lipoic acid** in cultured endothelial cells.
SO Diabetes, (1997) 46/9 (1481-1490).
Refs: 54
ISSN: 0012-1797 CODEN: DIAEAZ
AB . . . cultured bovine aortic endothelial cells (BAECs) with AGE albumin (500 nmol/l) resulted in the impairment of reduced glutathione (GSH) and **ascorbic acid** levels. As a consequence, increased cellular oxidative stress led to the activation of the transcription factor NF-.kappa.B and thus promoted. . . of various NF-.kappa.B-controlled genes, including endothelial tissue factor. Supplementation of the cellular antioxidative defense with the natural occurring antioxidant **.alpha.- lipoic acid** before AGE albumin induction completely prevented the AGE albumin-dependent depletion of reduced glutathione and **ascorbic acid**. Electrophoretic mobility shift assays (EMSAs) revealed that AGE albumin- mediated NF-.kappa.B activation was also reduced in a time- and dose-dependent manner as long as **.alpha.-lipoic acid** was added at least 30 min before AGE albumin stimulation. Inhibition was not due to physical interactions with **protein** DNA binding, since **.alpha.-lipoic acid**, directly included into the binding reaction, did not prevent binding activity of recombinant NF-.kappa.B. Western blots further demonstrated that **.alpha.-lipoic acid** inhibited the release and translocation of NF-.kappa.B from the cytoplasm into the nucleus. As a consequence, **.alpha.-lipoic acid** reduced AGE albumin-induced NF-.kappa.B mediated transcription and expression of endothelial genes relevant in diabetes, such as tissue factor and endothelin- 1. Thus, supplementation of cellular antioxidative defense mechanisms by extracellularly administered **.alpha.-**

lipoic acid reduces AGE albumin-induced endothelial dysfunction in vitro.

CT Medical Descriptors:
*diabetic angiopathy: ET, etiology
*diabetic angiopathy: CO, complication
angiogenesis
animal cell
antioxidant activity
aorta
article
cattle
cell culture
controlled study
diabetes mellitus
dna binding
endothelium cell
gene expression regulation
nonhuman
priority journal
protein dna interaction
***immunoglobulin enhancer binding protein**
*thioctic acid
albumin
antioxidant
ascorbic acid
glutathione
oxygen radical

RN (thioctic acid) 1077-29-8, 1200-22-2, 2319-84-8, 62-46-4; (**ascorbic acid**) 134-03-2, 15421-15-5, 50-81-7; (glutathione) 70-18-8

AB Depletion of cellular antioxidant defense mechanisms and the generation of oxygen free radicals by advanced glycation end products (AGEs) have been proposed to play a major role in the pathogenesis of diabetic vascular complications. Here we demonstrate that incubation of cultured bovine aortic endothelial cells (BAECs) with AGE albumin (500 nmol/l) resulted in the impairment of reduced glutathione (GSH) and **ascorbic acid** levels. As a consequence, increased cellular oxidative stress led to the activation of the transcription factor NF-.kappa.B and thus promoted the upregulation of various NF- .kappa.B-controlled genes, including endothelial tissue factor. Supplementation of the cellular antioxidative defense with the natural occurring antioxidant . **alpha.- lipoic acid** before AGE albumin induction completely prevented the AGE albumin-dependent depletion of reduced glutathione and **ascorbic acid**. Electrophoretic mobility shift assays (EMSAs) revealed that AGE albumin- mediated NF-.kappa.B activation was also reduced in a time- and dose-dependent manner as long as **.alpha.-lipoic acid** was added at least 30 min before AGE albumin stimulation. Inhibition was not due to physical interactions with **protein** DNA binding, since . **alpha.-lipoic acid**, directly included into the binding reaction, did not prevent binding activity of recombinant NF-.kappa.B. Western blots further demonstrated that **.alpha.-lipoic acid** inhibited the release and translocation of NF-.kappa.B from the cytoplasm into the nucleus. As a consequence, . **alpha.-lipoic acid** reduced AGE albumin-induced NF-.kappa.B mediated transcription and expression of endothelial genes relevant in diabetes, such as tissue factor and endothelin- 1. Thus, supplementation of cellular antioxidative defense mechanisms by extracellularly administered **.alpha.-lipoic acid** reduces AGE albumin-induced endothelial dysfunction in vitro.

AN 97265656 EMBASE

DN 1997265656

TI Advanced glycation end product-induced activation of NF-.kappa.B is suppressed by **.alpha.-lipoic acid** in

cultured endothelial cells.
AU Bierhaus A.; Chevion S.; Chevion M.; Hofmann M.; Quehenberger P.; Illmet T.; Luther T.; Berentshtein E.; Tritschler H.; Muller M.; Wahl P.; Ziegler R.; Nawroth P.P.
CS Dr. P.P. Nawroth, Medizinische Klinik I, Bergheimer Strasse 58, 69115 Heidelberg, Germany
SO Diabetes, (1997) 46/9 (1481-1490).
Refs: 54
ISSN: 0012-1797 CODEN: DIAEAZ
CY United States
DT Journal; Article
FS 003 Endocrinology
LA English
SL English

L7 ANSWER 8 OF 82 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
TI Neuroprotection by the metabolic antioxidant .**alpha**.-**lipoic acid**.
SO Free Radical Biology and Medicine, (1996) 22/1-2 (359-378).
ISSN: 0891-5849 CODEN: FRBMEH
AB . . . neural disorders involving free radical processes. Examination of current research reveals protective effects of these compounds in cerebral ischemia-reperfusion, excitotoxic **amino acid** brain injury, mitochondrial dysfunction, diabetes and diabetic neuropathy, inborn errors of metabolism, and other causes of acute or chronic damage. . . central to antioxidant defense in brain and other tissues. The most important thiol antioxidant, glutathione, cannot be directly administered, whereas .**alpha**.-**lipoic acid** can. In vitro, animal, and preliminary human studies indicate that .alpha.- lipoate may be effective in numerous neurodegenerative disorders.
CT Medical Descriptors:
*antioxidant activity
*degenerative disease
*ischemia
*neuroprotection
article
blood brain barrier
electron transport
hypothesis
neurotransmission
oxidative stress
priority journal
*thioctic acid
alpha tocopherol
ascorbic acid
dihydrolipoate
excitatory amino acid
neurotransmitter
RN (thioctic acid) 1077-29-8, 1200-22-2, 2319-84-8, 62-46-4; (alpha **tocopherol**) 1406-18-4, 1406-70-8, 52225-20-4, 58-95-7, 59-02-9; (**ascorbic acid**) 134-03-2, 15421-15-5, 50-81-7; (dihydrolipoate) 462-20-4
AB Reactive oxygen species are thought to be involved in a number of types of acute and chronic pathologic conditions in the brain and neural tissue. The metabolic antioxidant .alpha.-lipoate (thioctic acid, 1, 2-dithiolane-3- pentanoic acid; 1, 2-dithiolane-3 valeric acid; and 6,8-dithiooctanoic acid) is a low molecular weight substance that is absorbed from the diet and crosses the blood-brain barrier. .alpha.-Lipoate is taken up and reduced in cells and tissues to dihydrolipoate, which is also exported to the extracellular medium; hence,

protection is afforded to both intracellular and extracellular environments. Both .alpha.-lipoate and especially dihydrolipoate have been shown to be potent antioxidants, to regenerate through redox cycling other antioxidants like vitamin C and vitamin E, and to raise intracellular glutathione levels. Thus, it would seem an ideal substance in the treatment of oxidative brain and neural disorders involving free radical processes. Examination of current research reveals protective effects of these compounds in cerebral ischemia-reperfusion, excitotoxic **amino acid** brain injury, mitochondrial dysfunction, diabetes and diabetic neuropathy, inborn errors of metabolism, and other causes of acute or chronic damage to brain or neural tissue. Very few neuropharmacological intervention strategies are currently available for the treatment of stroke and numerous other brain disorders involving free radical injury. We propose that the various metabolic antioxidant properties of .alpha.-lipoate relate to its possible therapeutic roles in a variety of brain and neuronal tissue pathologies: thiols are central to antioxidant defense in brain and other tissues. The most important thiol antioxidant, glutathione, cannot be directly administered, whereas . **alpha.-lipoic acid** can. In vitro, animal, and preliminary human studies indicate that .alpha.- lipoate may be effective in numerous neurodegenerative disorders.

AN 96364277 EMBASE
DN 1996364277
TI Neuroprotection by the metabolic antioxidant **.alpha.-lipoic acid.**
AU Packer L.; Tritschler H.J.; Wessel K.
CS Department of Molecular/Cell Biology, 251 Life Sciences Addition, University of California,Berkeley, CA 94720-3200, United States
SO Free Radical Biology and Medicine, (1996) 22/1-2 (359-378).
ISSN: 0891-5849 CODEN: FRBMEH
CY United States
DT Journal; Article
FS 008 Neurology and Neurosurgery
021 Developmental Biology and Teratology
LA English
SL English

L7 ANSWER 9 OF 82 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
TI Effect of DL **.alpha.-lipoic acid** on tissue redox state in acute cadmium-challenged tissues.
SO Journal of Nutritional Biochemistry, (1996) 7/2 (85-92).
ISSN: 0955-2863 CODEN: JNBIEL
AB . . . contributing to the thiol pool of the cell. The present study was designed to determine whether dietary supplementation of DT **.alpha.-lipoic acid** (15 and 30 mg/kg), a 'meta-vitamin', to cadmium-intoxicated rats (3 mg/kg) affords protection against the oxidative stress caused by the. . . rats showed elevated levels of hydroxyl radicals and malondialdehyde (basal and induced), a decreased level of antioxidants-reduced glutathione, total thiols, **protein** thiols, nonprotein thiols, ascorbate, .alpha.-**tocopherol** and retinol and antioxidizing enzymes-superoxide dismutase, catalase, .tau.-glutamyl transpeptidase, glutathione peroxidase, glucose-6-phosphate dehydrogenase, glutathione reductase, and glutathione-S-transferase. Lipoate supplementation changed. . . indirectly by bolstering the antioxidants and antioxidizing enzyme defenses. In vitro studies revealed that, among the mono and dithiols (glutathione, **cysteine**, dithiothreitol, and lipoic acid), lipoic acid was the most potent scavenger of free radicals produced during cadmium-induced hepatotoxicity.
The drug. . .
CT Medical Descriptors:
*intoxication: . . . prevention

*intoxication: DT, drug therapy
*oxidative stress
animal model
article
controlled study
histochemistry
intraperitoneal drug administration
kidney
lipid peroxidation
liver
male
nonhuman
rat
*cadmium: TO, drug toxicity
*thioctic acid: DT, drug therapy
*thioctic acid: PD, pharmacology
alpha tocopherol: EC, endogenous compound
ascorbic acid: EC, endogenous compound
catalase: EC, endogenous compound
gamma glutamyltransferase: EC, endogenous compound
glucose 6 phosphate dehydrogenase: EC, endogenous compound
glutathione: EC, endogenous compound
glutathione. . .

RN (cadmium) 22537-48-0, 7440-43-9; (thioctic acid) 1077-29-8, 1200-22-2, 2319-84-8, 62-46-4; (alpha **tocopherol**) 1406-18-4, 1406-70-8, 52225-20-4, 58-95-7, 59-02-9; (**ascorbic acid**) 134-03-2, 15421-15-5, 50-81-7; (catalase) 9001-05-2; (gamma glutamyltransferase) 85876-02-4; (glucose 6 phosphate dehydrogenase) 37259-83-9, 9001-40-5; (glutathione) 70-18-8; (glutathione peroxidase) 9013-66-5; (glutathione. . .

AB Cadmium as an environmental pollutant has aroused great concern due to its toxic effects on various body tissues. Supplementation of thiol compounds has been suggested to protect against the toxic effects of reduced oxygen species by contributing to the thiol pool of the cell. The present study was designed to determine whether dietary supplementation of DT . **alpha.-lipoic acid** (15 and 30 mg/kg), a 'meta-vitamin', to cadmium-intoxicated rats (3 mg/kg) affords protection against the oxidative stress caused by the metal. The liver and kidney of the metal-administered rats showed elevated levels of hydroxyl radicals and malondialdehyde (basal and induced), a decreased level of antioxidants-reduced glutathione, total thiols, **protein** thiols, nonprotein thiols, ascorbate, .alpha.-**tocopherol** and retinol and antioxidizing enzymes-superoxide dismutase, catalase, .tau.-glutamyl transpeptidase, glutathione peroxidase, glucose-6-phosphate dehydrogenase, glutathione reductase, and glutathione-S-transferase. Lipoate supplementation changed the tissue redox state directly by scavenging the free radicals and indirectly by bolstering the antioxidants and antioxidizing enzyme defenses. In vitro studies revealed that, among the mono and dithiols (glutathione, **cysteine**, dithiothreitol, and lipoic acid), lipoic acid was the most potent scavenger of free radicals produced during cadmium-induced hepatotoxicity. The drug contributes its thiol groups to detoxify the divalent metal and subsequently ameliorates the cell membrane integrity.

AN 96079987 EMBASE
DN 1996079987

TI Effect of DL **.alpha.-lipoic acid** on tissue redox state in acute cadmium-challenged tissues.

AU Sumathi R.; Baskaran G.; Varalakshmi P.
CS Department of Medical Biochemistry, Dr. A.L. Mudaliar Post Grad. Inst., Basic Medical Sciences,Taramani, Madras 600 113, India

SO Journal of Nutritional Biochemistry, (1996) 7/2 (85-92).
ISSN: 0955-2863 CODEN: JNBIEL
CY United States
DT Journal; Article

FS 029 Clinical Biochemistry
052 Toxicology
030 Pharmacology
037 Drug Literature Index
LA English
SL English

L7 ANSWER 10 OF 82 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
TI **.alpha.-Lipoic acid** prevents buthionine sulfoximine-induced cataract formation in newborn rats.
SO Free Radical Biology and Medicine, (1995) 18/4 (823-829).
ISSN: 0891-5849 CODEN: FRBMEH
AB We investigated the effect of **.alpha.-lipoic acid**, a powerful antioxidant, on cataract formation in L-buthionine(S,R)sulfoximine (BSO)-treated newborn rats and found that a dose of 25 mg/kg b.w.. . . therapeutic antioxidants in protecting animals from cataract formation. Major biochemical changes in the lens associated with the protective effect of **.alpha.-lipoic acid** were increases in glutathione, ascorbate, and vitamin E levels, loss of which are effects of BSO administration. Treatment with **.alpha.-lipoic acid** also restored the activities of glutathione peroxidase, catalase, and ascorbate free radical reductase in lenses of L-buthionine(S,R)-sulfoximine-treated animals but did not affect glutathione reductase or superoxide dismutase activity. We conclude that **.alpha.-lipoic acid** may take over some of the functions of glutathione (e.g., maintaining the higher level of ascorbate, indirect participation in vitamin. . . the increase of glutathione level in lens tissue mediated by lipoate could be also due to a direct protection of **protein** thiols. Thus, **.alpha.-lipoic acid** could be of potential therapeutic use in preventing cataracts and their complications.
CT Medical Descriptors:
*cataractogenesis
animal . . .
drug toxicity
*thioctic acid: PD, pharmacology
*thioctic acid: CB, drug combination
*thioctic acid: IT, drug interaction
*thioctic acid: DO, drug dose
*thioctic acid: DT, drug therapy
alpha tocopherol: EC, endogenous compound
ascorbic acid: EC, endogenous compound
catalase: EC, endogenous compound
glutamate cysteine ligase: EC, endogenous compound
glutathione: EC, endogenous compound
glutathione peroxidase: EC, endogenous compound
glutathione reductase: EC, endogenous compound
superoxide dismutase: EC, endogenous compound
RN (buthionine sulfoximine) 5072-26-4; (thioctic acid) 1077-29-8, 1200-22-2, 2319-84-8, 62-46-4; (alpha **tocopherol**) 1406-18-4, 1406-70-8, 52225-20-4, 58-95-7, 59-02-9; (**ascorbic acid**) 134-03-2, 15421-15-5, 50-81-7; (catalase) 9001-05-2; (glutamate **cysteine** ligase) 9023-64-7; (glutathione) 70-18-8; (glutathione peroxidase) 9013-66-5; (glutathione reductase) 9001-48-3; (superoxide dismutase) 37294-21-6, 9016-01-7, 9054-89-1
AB We investigated the effect of **.alpha.-lipoic acid**, a powerful antioxidant, on cataract formation in L-buthionine(S,R)sulfoximine (BSO)-treated newborn rats and found that a dose of 25 mg/kg b.w. protected 60% of animals from cataract formation. L-buthionine(S,R)-sulfoximine is an inhibitor of glutathione synthesis, whose administration to newborn animals leads to the development of cataracts; this is a potential model for studying the role of therapeutic antioxidants in protecting animals from cataract formation. Major biochemical changes in the lens associated with the protective effect of

alpha.-lipoic acid were increases in glutathione, ascorbate, and vitamin E levels, loss of which are effects of BSO administration. Treatment with **.alpha.-lipoic acid** also restored the activities of glutathione peroxidase, catalase, and ascorbate free radical reductase in lenses of L-buthionine(S,R)-sulfoximine-treated animals but did not affect glutathione reductase or superoxide dismutase activity. We conclude that **alpha.-lipoic acid** may take over some of the functions of glutathione (e.g., maintaining the higher level of ascorbate, indirect participation in vitamin E recycling); the increase of glutathione level in lens tissue mediated by lipoate could be also due to a direct protection of **protein** thiols. Thus, **.alpha.-lipoic acid** could be of potential therapeutic use in preventing cataracts and their complications.

AN 95077365 EMBASE
DN 1995077365
TI **.alpha.-Lipoic acid** prevents buthionine sulfoximine-induced cataract formation in newborn rats.
AU Maitra I.; Serbinova E.; Trischler H.; Packer L.
CS Department Molecular/Cell Biology, University of California,Berkeley, CA 94720, United States
SO Free Radical Biology and Medicine, (1995) 18/4 (823-829). ISSN: 0891-5849 CODEN: FRBMEH
CY United States
DT Journal; Article
FS 012 Ophthalmology
029 Clinical Biochemistry
030 Pharmacology
037 Drug Literature Index
LA English
SL English

=> d 11-82 kwic bib

L7 ANSWER 11 OF 82 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
TI Effect of **.alpha.-lipoic acid** on the peripheral conversion of thyroxine to triiodothyronine and on serum lipid-, **protein-** and glucose levels.
SO Arzneimittel-Forschung/Drug Research, (1991) 41/12 (1294-1298). ISSN: 0004-4172 CODEN: ARZNAD
AB The influence of **.alpha.-lipoic acid** (LA, thioctic acid, CAS 62-46-4) on thyroid hormone metabolism and serum lipid-, **protein-** and glucose levels was investigated. In the first setup of experiments administration of LA together with thyroxine (T4) for 9. . . LA decreased the triglyceride level by 45%; the decrease induced by T4 or LA plus T4 was not significant. Total **protein** and albumin levels decreased by LA plus T4 treatment when compared to the LA control. The slight increase in glucose. . . 30%, and LA plus T4 further reduced it by 47%. The triglycerides were not affected. A moderate decrease in total **protein** was observed after treatment with T4 plus LA; T4 and LA plus T4 decreased the albumin level. The decrease in. . . of LA on the peripheral conversion of T4-to-T3. LA with T4 exerts a lipid lowering effect and minimal effects on **protein** and **carbohydrate** metabolism.
CT Medical Descriptors:
*metabolism
animal . . .
interaction
*thyroxine: PK, pharmacokinetics
*thyroxine: CB, drug combination

albumin: EC, endogenous compound
cholesterol: EC, endogenous compound
glucose: EC, endogenous compound
liothyronine: EC, endogenous compound
propylthiouracil: EC, endogenous compound
protein: EC, endogenous compound
triacylglycerol: EC, endogenous compound
RN (thioctic acid) 1077-29-8, 1200-22-2, 2319-84-8, 62-46-4; (thyroxine) 7488-70-2; (cholesterol) 57-88-5; (glucose) 50-99-7, 84778-64-3; (liothyronine) 6138-47-2, 6893-02-3; (propylthiouracil) 51-52-5; (**protein**) 67254-75-5
AN 92010320 EMBASE
DN 1992010320
TI Effect of **.alpha.-lipoic acid** on the peripheral conversion of thyroxine to triiodothyronine and on serum lipid-, **protein-** and glucose levels.
AU Segermann J.; Hotze A.; Ulrich H.; Rao G.S.
CS Institut fur Klinische, Biochemie, Universitat Bonn, Sigmund-Freud-Strasse 25,W-5300 Bonn, Germany
SO Arzneimittel-Forschung/Drug Research, (1991) 41/12 (1294-1298). ISSN: 0004-4172 CODEN: ARZNAD
CY Germany
DT Journal; Article
FS 029 Clinical Biochemistry
030 Pharmacology
037 Drug Literature Index
LA English
SL English; German

L7 ANSWER 12 OF 82 BIOSIS COPYRIGHT 2001 BIOSIS
SO Biochemical Pharmacology, (**June 1, 1998**) Vol. 55, No. 11, pp. 1747-1758.
ISSN: 0006-2952.
AB. . . a fundamental regulatory mechanism in cell biology. Electron flow through side chain functional CH2-SH groups of conserved cysteinyl residues in **proteins** account for their redox-sensing properties. Because in most intracellular **proteins** thiol groups are strongly "buffered" against oxidation by the highly reduced environment inside the cell, only accessible **protein** thiol groups with high thiol-disulfide oxidation potentials are likely to be redox sensitive. The list of redox-sensitive signal transduction pathways. . . are of central importance in redox signaling. Among the thiol agents tested for their efficacy to modulate cellular redox status, N-acetyl-L-**cysteine** (NAC) and **alpha-lipoic acid** hold promise for clinical use. A unique advantage of lipoate is that it is able to utilize cellular reducing equivalents,. . . regenerate its reductive vicinal dithiol form. Because lipoate can be readily recycled in the cell, it has an advantage over N-acetyl-L-**cysteine** on a concentration:effect basis. Our current knowledge of redox regulated signal transduction has led to the unfolding of the remarkable. . .
IT . . .
Metabolism; Pharmacology
IT Diseases
cancer: neoplastic disease; AIDS [acquired immunodeficiency syndrome]: immune system disease, viral disease
IT Chemicals & Biochemicals
alpha-lipoic acid; glutathione; thiol antioxidants; thioredoxin; N-acetyl-L-**cysteine**
RN 70-18-8 (GLUTATHIONE)
616-91-1 (N-ACETYL-L-**CYSTEINE**)
1200-22-2 (**ALPHA-LIPOIC ACID**)
AN 1998:323315 BIOSIS

DN PREV199800323315
TI Redox signaling and the emerging therapeutic potential of the thiol antioxidants.
AU Sen, Chandan K. (1)
CS (1) 251 Life Sci. Addition, Dep. Mol. Cell Biol., Univ. California, Berkeley, CA 94720 USA
SO Biochemical Pharmacology, (**June 1, 1998**) Vol. 55, No. 11, pp. 1747-1758.
ISSN: 0006-2952.
DT General Review
LA English

L7 ANSWER 13 OF 82 BIOSIS COPYRIGHT 2001 BIOSIS
TI **alpha-Lipoic acid** in liver metabolism and disease.
SO Free Radical Biology & Medicine, (**April, 1998**) Vol. 24, No. 6, pp. 1023-1039.
ISSN: 0891-5849.
AB R-**alpha-Lipoic acid** is found naturally occurring as a prosthetic group in alpha-keto acid dehydrogenase complexes of the mitochondria, and as such plays a fundamental role in metabolism. Although this has been known for decades, only recently has free supplemented **alpha-lipoic acid** been found to affect cellular metabolic processes in vitro, as it has the ability to alter the redox status of. . . it appears that this compound has important therapeutic potential in conditions where oxidative stress is involved. Early case studies with **alpha-lipoic acid** were performed with little knowledge of the action of **alpha-lipoic acid** at a cellular level, but with the rationale that because the naturally occurring **protein** bound form of **alpha-lipoic acid** has a pivotal role in metabolism, that supplementation may have some beneficial effect. Such studies sought to evaluate the effect of supplemented **alpha-lipoic acid**, using low doses, on lipid or **carbohydrate** metabolism, but little or no effect was observed. A common response in these trials was an increase in glucose uptake,. . . lactate were also observed, suggesting that an inhibitory effect on the pyruvate dehydrogenase complex was occurring. During the same period, **alpha-lipoic acid** was also used as a therapeutic agent in a number of conditions relating to liver disease, including alcohol-induced damage, mushroom poisoning, metal intoxification, and CCl4 poisoning. **alpha-Lipoic acid** supplementation was successful in the treatment for these conditions in many cases. Experimental studies and clinical trials in the last 5 years using high doses of **alpha-lipoic acid** (600 mg in humans) have provided new and consistent evidence for the therapeutic role of antioxidant **alpha-lipoic acid** in the treatment of insulin resistance and diabetic polyneuropathy. This new insight should encourage clinicians to use **alpha-lipoic acid** in diseases affecting liver in which oxidative stress is involved.
IT . . .
nervous system disease, metabolic disease; liver damage: digestive system disease; primary biliary cirrhosis: digestive system disease
IT Chemicals & Biochemicals
alpha-lipoic acid: antioxidant, dietary supplement; free radicals; glucose; lactate: plasma; pyruvate dehydrogenase complex; pyruvate: plasma
IT Methods & Equipment
dietary therapy: therapeutic method
IT Miscellaneous Descriptors
carbohydrate metabolism; lipid metabolism; oxidative stress
RN 1200-22-2 (**ALPHA-LIPOIC ACID**)
50-99-7Q (GLUCOSE)
58367-01-4Q (GLUCOSE)

57-60-3 (PYRUVATE)
113-21-3 (LACTATE)
9014-20-4 (PYRUVATE DEHYDROGENASE COMPLEX)
AN 1998:268911 BIOSIS
DN PREV199800268911
TI **alpha-Lipoic acid** in liver metabolism and disease.
AU Bustamante, Juanita (1); Lodge, John K. (1); Marcocci, Lucia (1); Tritschler, Hans J.; Packer, Lester (1); Rihn, Bertrand H. (1)
CS (1) Membranes Bioenergetics Group, Dep. Molecular Cell Biol., Univ. California, Berkeley, CA USA
SO Free Radical Biology & Medicine, (**April, 1998**) Vol. 24, No. 6, pp. 1023-1039.
ISSN: 0891-5849.
DT Article
LA English

L7 ANSWER 14 OF 82 BIOSIS COPYRIGHT 2001 BIOSIS
SO Biochemistry and Molecular Biology International, (**Sept., 1997**) Vol. 42, No. 6, pp. 1189-1197.
ISSN: 1039-9712.
AB The effect of several antioxidants and **cysteine**-elevating precursor drugs (prodrugs) was tested on lens damage occurring after in vitro exposure to low levels of 60Co-gamma-irradiation, to simulate in vitro the exposure to radiation in vivo of (1) astronauts (2) jet crews (3) military radiation accident personnel. **Tocopherol** (100 muM), **ascorbic acid** (1 mM), R-**alpha-lipoic acid** (1 mM), and taurine (0.5 mM) protected against radiation-associated **protein** leakage. MTCA and ribocysteine protected lenses against opacification, LDH and **protein** leakage, indicating that antioxidants and prodrugs of **cysteine** appear to offer protection against lens damage caused by low level radiation.
IT . . .
and Molecular Biophysics; Ophthalmology (Human Medicine, Medical Sciences); Radiation Biology
IT Diseases
cataract: eye disease
IT Chemicals & Biochemicals
antioxidants; **ascorbic acid**: antioxidant; taurine: antioxidant; **tocopherol**: antioxidant; R-**alpha-lipoic acid**: antioxidant
IT Miscellaneous Descriptors
aging; lens opacification; oxidative stress; **protein** leakage; radiation; space environment; terrestrial environment
RN 50-81-7Q (**ASCORBIC ACID**)
62624-30-0Q (**ASCORBIC ACID**)
107-35-7 (TAURINE)
AN 1998:222826 BIOSIS
DN PREV199800222826
TI Antioxidants and cataract: (Cataract induction in space environment and application to terrestrial aging cataract.
AU Bantseev, Vladimir (1); Bhardwaj, Ratan; Rathbun, W.; Hagasawa, H.; Trevithick, John R.
CS (1) Dep. Biochemistry, Univ. Western Ontario, London, ON N6A 5C1 Canada
SO Biochemistry and Molecular Biology International, (**Sept., 1997**) Vol. 42, No. 6, pp. 1189-1197.
ISSN: 1039-9712.
DT Article
LA English

L7 ANSWER 15 OF 82 BIOSIS COPYRIGHT 2001 BIOSIS
TI Advanced glycation end product-induced activation of NF-kappa-B is suppressed by **alpha-lipoic acid** in cultured endothelial cells.
SO Diabetes, (1997) Vol. 46, No. 9, pp. 1481-1490.
ISSN: 0012-1797.

AB. . . cultured bovine aortic endothelial cells (BAECs) with AGE albumin (500 nmol/l) resulted in the impairment of reduced glutathione (GSH) and **ascorbic acid** levels. As a consequence, increased cellular oxidative stress led to the activation of the transcription factor NF-kappa-B and thus promoted. . . upregulation of various NF-kappa-B-controlled genes, including endothelial tissue factor. Supplementation of the cellular antioxidative defense with the natural occurring antioxidant **alpha-lipoic acid** before AGE albumin induction completely prevented the AGE albumin-dependent depletion of reduced glutathione and **ascorbic acid**. Electrophoretic mobility shift assays (EMSAs) revealed that AGE albumin-mediated NF-kappa-B activation was also reduced in a time- and dose-dependent manner as long as **alpha-lipoic acid** was added at least 30 min before AGE albumin stimulation. Inhibition was not due to physical interactions with **protein** DNA binding, since **alpha-lipoic acid**, directly included into the binding reaction, did not prevent binding activity of recombinant NF-kappa-B. Western blots further demonstrated that **alpha-lipoic acid** inhibited the release and translocation of NF-kappa-B from the cytoplasm into the nucleus. As a consequence, **alpha-lipoic acid** reduced AGE albumin-induced NF-kappa-B mediated transcription and expression of endothelial genes relevant in diabetes, such as tissue factor and endothelin-1. Thus, supplementation of cellular antioxidative defense mechanisms by extracellularly administered **alpha-lipoic acid** reduces AGE albumin-induced endothelial dysfunction in vitro.

IT . . .
Molecular Biophysics); Cardiovascular System (Transport and Circulation); Cell Biology; Endocrine System (Chemical Coordination and Homeostasis); Metabolism

IT Chemicals & Biochemicals
ALPHA-LIPOIC ACID; GLUTATHIONE; **ASCORBIC ACID**

IT Miscellaneous Descriptors
ACTIVATION; ADVANCED GLYCATION ENDPRODUCTS; **ALPHA-LIPOIC ACID**; ANTIOXIDANT; **ASCORBIC ACID**; CARDIOVASCULAR SYSTEM; CIRCULATORY SYSTEM; DIABETIC VASCULAR COMPLICATIONS; DNA; ENDOCRINE DISEASE/PANCREAS; ENDOCRINE SYSTEM; ENDOTHELIAL CELLS; ENDOTHELIN-1; GLUTATHIONE; NUCLEAR FACTOR-KAPPA-B; PATHOGENESIS; **PROTEIN**-BINDING; VASCULAR DISEASE

RN 1200-22-2 (**ALPHA-LIPOIC ACID**)
70-18-8 (GLUTATHIONE)
50-81-7 (**ASCORBIC ACID**)

AN 1997:437275 BIOSIS

DN PREV199799736478

TI Advanced glycation end product-induced activation of NF-kappa-B is suppressed by **alpha-lipoic acid** in cultured endothelial cells.

AU Bierhaus, Angelika; Chevion, Shlomit; Chevion, Mordechai; Hofmann, Marion; Quehenberger, Peter; Illmer, Thomas; Luther, Thomas; Berentshtein, Eduard; Tritschler, Hans; Muller, Martin; Wahl, Peter; Ziegler, Reinhard; Nawroth, Peter P. (1)

CS (1) Medizinische Klinik I, Bergheimer Strasse 58, 69115 Heidelberg Germany

SO Diabetes, (1997) Vol. 46, No. 9, pp. 1481-1490.
ISSN: 0012-1797.

DT Article

LA English

L7 ANSWER 16 OF 82 BIOSIS COPYRIGHT 2001 BIOSIS

TI Effect of DL **alpha-lipoic acid** on tissue redox state in acute cadmium-challenged tissues.
SO Journal of Nutritional Biochemistry, (1996) Vol. 7, No. 2, pp. 85-92. ISSN: 0955-2863.
AB. . . contributing to the thiol pool of the cell. The present study was designed to determine whether dietary supplementation of DL **alpha -lipoic acid** (15 and 30 mg/kg), a "meta-vitamin," to cadmium-intoxicated rats (3 mg/kg) affords protection against the oxidative stress caused by the. . . rats showed elevated levels of hydroxyl radicals and malondialdehyde (basal and induced), a decreased level of antioxidants-reduced glutathione, total thiols, **protein** thiols, nonprotein thiols, ascorbate, alpha-**tocopherol** and retinol and antioxidizing enzymes-superoxide dismutase, catalase, tau-glutamyl transpeptidase, glutathione peroxidase, glucose-6-phosphate dehydrogenase, glutathione reductase, and glutathione-S-transferase. Lipoate supplementation changed. . . indirectly by bolstering the antioxidants and antioxidizing enzyme defenses. In vitro studies revealed that, among the mono and dithiols (glutathione, **cysteine**, dithiothreitol, and lipoic acid), lipoic acid was the most potent scavenger of free radicals produced during cadmium-induced hepatotoxicity.
The drug. . .
IT . . .
Nutrition; Pathology; Physiology; Pollution Assessment Control and Management; Toxicology; Urinary System (Chemical Coordination and Homeostasis)
IT Chemicals & Biochemicals
DL **ALPHA-LIPOIC ACID**
RN 1077-28-7 (DL **ALPHA-LIPOIC ACID**)
AN 1996:196194 BIOSIS
DN PREV199698752323
TI Effect of DL **alpha-lipoic acid** on tissue redox state in acute cadmium-challenged tissues.
AU Sumathi, Ramachandran; Baskaran, Govindarajan; Varalakshmi, Palaninathan (1)
CS (1) Dep. Med. Biochem., Dr. A. L. Mudaliar Post Grad. Inst. Basic Med. Sci., Taramani, Madras 600 113 India
SO Journal of Nutritional Biochemistry, (1996) Vol. 7, No. 2, pp. 85-92. ISSN: 0955-2863.
DT Article
LA English

L7 ANSWER 17 OF 82 BIOSIS COPYRIGHT 2001 BIOSIS
TI EFFECT OF **ALPHA LIPOIC ACID** ON THE PERIPHERAL CONVERSION OF THYROXINE TO TRIIODOTHYRONINE AND ON SERUM LIPID **PROTEIN** AND GLUCOSE LEVELS.
SO ARZNEIM-FORSCH, (1991) 41 (12), 1294-1298. CODEN: ARZNAD. ISSN: 0004-4172.
AB The influence of **.alpha.-lipoic acid** (LA, thioctic acid, CAS 62-46-4) on thyroid hormone metabolism and serum lipid-, **protein**- and glucose levels was investigated. In the first setup of experiments administration of LA together with thyroxine (T4) for 9. . . LA decreased the triglyceride level by 45%; the decrease induced by T4 or LA plus T4 was not significant. Total **protein** and albumin levels decreased by LA plus T4 treatment when compared to the LA control. The slight increase in glucose. . . 30%, and LA plus T4 further reduced it by 47%. The triglycerides were not affected. A moderate decrease in total **protein** was observed after treatment with T4 plus LA:T4 and LA plus T4 decreased the albumin level. The decrease in serum. . . of LA on the peripheral conversion of T4-to-T3. LA with T4 exerts a lipid lowering effect and minimal effects on **protein** and **carbohydrate** metabolism.
RN 50-99-7 (GLUCOSE)
51-48-9 (THYROXINE)

57-88-5 (CHOLESTEROL)
1200-22-2 (**ALPHA LIPOIC ACID**)
6893-02-3 (TRIIODOTHYRONINE)
AN 1992:122779 BIOSIS
DN BA93:68579
TI EFFECT OF **ALPHA LIPOIC ACID** ON THE PERIPHERAL CONVERSION OF THYROXINE TO TRIIODOTHYRONINE AND ON SERUM LIPID **PROTEIN** AND GLUCOSE LEVELS.
AU SEGERMANN J; HOTZE A; ULRICH H; RAO G S
CS INST. FUER KLINISCHE BIOCHEMIE, UNIVERSITAET, SIGMUND-FREUD-STRASSE 25, W-5300 BONN, GERMANY.
SO ARZNEIM-FORSCH, (1991) 41 (12), 1294-1298.
CODEN: ARZNAD. ISSN: 0004-4172.
FS BA; OLD
LA English

L7 ANSWER 18 OF 82 MEDLINE
SO BIOCHEMICAL PHARMACOLOGY, **(1998 Jun 1)** 55 (11) 1747-58. Ref: 134
Journal code: 9Z4; 0101032. ISSN: 0006-2952.
AB . . . a fundamental regulatory mechanism in cell biology. Electron flow through side chain functional CH2-SH groups of conserved cysteinyl residues in **proteins** account for their redox-sensing properties. Because in most intracellular **proteins** thiol groups are strongly "buffered" against oxidation by the highly reduced environment inside the cell, only accessible **protein** thiol groups with high thiol-disulfide oxidation potentials are likely to be redox sensitive. The list of redox-sensitive signal transduction pathways. . . are of central importance in redox signaling. Among the thiol agents tested for their efficacy to modulate cellular redox status, N-acetyl-L-**cysteine** (NAC) and **alpha-lipoic acid** hold promise for clinical use. A unique advantage of lipoate is that it is able to utilize cellular reducing equivalents,. . . regenerate its reductive vicinal dithiol form. Because lipoate can be readily recycled in the cell, it has an advantage over N-acetyl-L-**cysteine** on a concentration:effect basis. Our current knowledge of redox regulated signal transduction has led to the unfolding of the remarkable. . .
CT Check Tags: Animal; Human
*Antioxidants: PD, pharmacology
Antioxidants: TU, therapeutic use
DNA-Binding Proteins: GE, genetics
DNA-Binding Proteins: ME, metabolism
Gene Expression Regulation
Glutathione: ME, metabolism
Oxidation-Reduction
Protein Binding
Reactive Oxygen Species: ME, metabolism
*Signal Transduction: DE, drug effects
Signal Transduction: GE, genetics
Signal Transduction: PH, physiology
. . .
CN 0 (Antioxidants); 0 (DNA-Binding **Proteins**); 0 (Reactive Oxygen Species); 0 (Sulfhydryl Compounds)
AN 1998378094 MEDLINE
DN 98378094 PubMed ID: 9714292
TI Redox signaling and the emerging therapeutic potential of thiol antioxidants.
AU Sen C K
CS Department of Molecular and Cell Biology, University of California, Berkeley 94720-3200, USA.. cksen@socrates.berkeley.edu
SO BIOCHEMICAL PHARMACOLOGY, **(1998 Jun 1)** 55 (11) 1747-58. Ref: 134

Journal code: 9Z4; 0101032. ISSN: 0006-2952.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LA English
FS Priority Journals
EM 199808
ED Entered STN: 19980910
Last Updated on STN: 19980910
Entered Medline: 19980831

L7 ANSWER 19 OF 82 MEDLINE
TI **Alpha-lipoic acid** in liver metabolism and disease.
SO FREE RADICAL BIOLOGY AND MEDICINE, **(1998 Apr)** 24 (6) 1023-39.
Ref: 141
Journal code: FRE; 8709159. ISSN: 0891-5849.
AB R-**alpha-Lipoic acid** is found naturally occurring as a prosthetic group in alpha-keto acid dehydrogenase complexes of the mitochondria, and as such plays a fundamental role in metabolism. Although this has been known for decades, only recently has free supplemented **alpha-lipoic acid** been found to affect cellular metabolic processes in vitro, as it has the ability to alter the redox status of. . . it appears that this compound has important therapeutic potential in conditions where oxidative stress is involved. Early case studies with **alpha-lipoic acid** were performed with little knowledge of the action of **alpha-lipoic acid** at a cellular level, but with the rationale that because the naturally occurring **protein** bound form of **alpha-lipoic acid** has a pivotal role in metabolism, that supplementation may have some beneficial effect. Such studies sought to evaluate the effect of supplemented **alpha-lipoic acid**, using low doses, on lipid or **carbohydrate** metabolism, but little or no effect was observed. A common response in these trials was an increase in glucose uptake,. . . lactate were also observed, suggesting that an inhibitory effect on the pyruvate dehydrogenase complex was occurring. During the same period, **alpha-lipoic acid** was also used as a therapeutic agent in a number of conditions relating to liver disease, including alcohol-induced damage, mushroom poisoning, metal intoxification, and CCl4 poisoning. **Alpha-Lipoic acid** supplementation was successful in the treatment for these conditions in many cases. Experimental studies and clinical trials in the last 5 years using high doses of **alpha-lipoic acid** (600 mg in humans) have provided new and consistent evidence for the therapeutic role of antioxidant **alpha-lipoic acid** in the treatment of insulin resistance and diabetic polyneuropathy. This new insight should encourage clinicians to use **alpha-lipoic acid** in diseases affecting liver in which oxidative stress is involved.
AN 1998268630 MEDLINE
DN 98268630 PubMed ID: 9607614
TI **Alpha-lipoic acid** in liver metabolism and disease.
AU Bustamante J; Lodge J K; Marcocci L; Tritschler H J; Packer L; Rihn B H
CS Department of Molecular and Cell Biology, University of California, Berkeley 94720-3200, USA.
NC DK 50430-01 (NIDDK)
SO FREE RADICAL BIOLOGY AND MEDICINE, **(1998 Apr)** 24 (6) 1023-39.
Ref: 141
Journal code: FRE; 8709159. ISSN: 0891-5849.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)

(REVIEW, TUTORIAL)
LA English
FS Priority Journals
EM 199810
ED Entered STN: 19990106
Last Updated on STN: 19990106
Entered Medline: 19981030

L7 ANSWER 20 OF 82 MEDLINE
SO BIOCHEMISTRY AND MOLECULAR BIOLOGY INTERNATIONAL, **(1997 Sep)** 42 (6) 1189-97.
Journal code: BOD; 9306673. ISSN: 1039-9712.
AB The effect of several antioxidants and **cysteine**-elevating precursor drugs (prodrugs) was tested on lens damage occurring after in vitro exposure to low levels of 60Co-gamma-irradiation, to simulate in vitro the exposure to radiation in vivo of (1) astronauts (2) jet crews (3) military radiation accident personnel. **Tocopherol** (100 microM), **ascorbic acid** (1 mM), R-**alpha-lipoic acid** (1 mM), and taurine (0.5 mM) protected against radiation-associated **protein** leakage. MTCA and ribocysteine protected lenses against opacification, LDH and **protein** leakage, indicating that antioxidants and prodrugs of **cysteine** appear to offer protection against lens damage caused by low level radiation.
CT Check Tags: Animal; In Vitro; Support, Non-U.S. Gov't
*Antioxidants: PD, pharmacology
Ascorbic Acid: PD, pharmacology
Carbolines: PD, pharmacology
*Cataract: PP, physiopathology
Cataract: RT, radiotherapy
Cysteine: AA, analogs & derivatives
Cysteine: PD, pharmacology
Dose-Response Relationship, Radiation
Lactate Dehydrogenase: DE, drug effects
Lactate Dehydrogenase: ME, metabolism
Lactate Dehydrogenase: RE, radiation effects
. . .
RN 107-35-7 (Taurine); 1406-18-4 (Vitamin E); 50-69-1 (Ribose); **50-81-7 (Ascorbic Acid); 52-90-4 (Cysteine)**; 5470-37-1 (1-methyl-1,2,3,4-tetrahydro-beta-carboline-3-carboxylic acid); 62-46-4 (Thioctic Acid)
AN 97450549 MEDLINE
DN 97450549 PubMed ID: 9305537
TI Antioxidants and cataract: (cataract induction in space environment and application to terrestrial aging cataract).
AU Bantseev V; Bhardwaj R; Rathbun W; Nagasawa H; Trevithick J R
CS Department of Biochemistry, University of Western Ontario, London, Canada.
SO BIOCHEMISTRY AND MOLECULAR BIOLOGY INTERNATIONAL, **(1997 Sep)** 42 (6) 1189-97.
Journal code: BOD; 9306673. ISSN: 1039-9712.
CY Australia
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199711
ED Entered STN: 19971224
Last Updated on STN: 20000303
Entered Medline: 19971103

L7 ANSWER 21 OF 82 MEDLINE
TI Advanced glycation end product-induced activation of NF-kappaB is suppressed by **alpha-lipoic acid** in cultured endothelial cells.
SO DIABETES, **(1997 Sep)** 46 (9) 1481-90.
Journal code: E8X; 0372763. ISSN: 0012-1797.

AB . . . cultured bovine aortic endothelial cells (BAECs) with AGE albumin
(500 nmol/l) resulted in the impairment of reduced glutathione (GSH) and **ascorbic acid** levels. As a consequence, increased cellular oxidative stress led to the activation of the transcription factor NF-kappaB and thus promoted. . . upregulation of various NF-kappaB-controlled genes, including endothelial tissue factor. Supplementation of the cellular antioxidative defense with the natural occurring antioxidant **alpha-lipoic acid** before AGE albumin induction completely prevented the AGE albumin-dependent depletion of reduced glutathione and **ascorbic acid**. Electrophoretic mobility shift assays (EMSAs) revealed that AGE albumin-mediated NF-kappaB activation was also reduced in a time- and dose-dependent manner as long as **alpha-lipoic acid** was added at least 30 min before AGE albumin stimulation. Inhibition was not due to physical interactions with **protein** DNA binding, since **alpha-lipoic acid**, directly included into the binding reaction, did not prevent binding activity of recombinant NF-kappaB. Western blots further demonstrated that **alpha-lipoic acid** inhibited the release and translocation of NF-kappaB from the cytoplasm into the nucleus. As a consequence, **alpha-lipoic acid** reduced AGE albumin-induced NF-kappaB mediated transcription and expression of endothelial genes relevant in diabetes, such as tissue factor and endothelin-1. Thus, supplementation of cellular antioxidative defense mechanisms by extracellularly administered **alpha-lipoic acid** reduces AGE albumin-induced endothelial dysfunction in vitro.

CT Check Tags: Human; Support, Non-U.S. Gov't
*Antioxidants: PD, pharmacology
Ascorbic Acid: CH, chemistry
Ascorbic Acid: ME, metabolism
Cell Compartmentation: DE, drug effects
Cell Nucleus: ME, metabolism
Cells, Cultured
Cytoplasm: ME, metabolism
DNA-Binding Proteins: ME, metabolism
*Endothelium, Vascular: PH, physiology
Gene Expression Regulation: DE, drug effects
Glutathione: ME, metabolism
*Glycosylation End Products,. . .

RN **50-81-7 (Ascorbic Acid)**; 62-46-4 (Thioctic Acid); 70-18-8 (Glutathione); 9035-58-9 (Thromboplastin)

CN 0 (Antioxidants); 0 (DNA-Binding **Proteins**); 0 (Glycosylation End Products, Advanced); 0 (NF-kappa B)

AN 97431545 MEDLINE

DN 97431545 PubMed ID: 9287050

TI Advanced glycation end product-induced activation of NF-kappaB is suppressed by **alpha-lipoic acid** in cultured endothelial cells.

AU Bierhaus A; Chevion S; Chevion M; Hofmann M; Quehenberger P; Illmer T; Luther T; Berentshtein E; Tritschler H; Muller M; Wahl P; Ziegler R; Nawroth P P

CS Department of Internal Medicine, University of Heidelberg, Germany.

SO DIABETES, **(1997 Sep)** 46 (9) 1481-90.
Journal code: E8X; 0372763. ISSN: 0012-1797.

CY United States

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Abridged Index Medicus Journals; Priority Journals

EM 199709

ED Entered STN: 19971008
Last Updated on STN: 19971008
Entered Medline: 19970925

L7 ANSWER 22 OF 82 MEDLINE

TI **Alpha-lipoic acid** prevents buthionine

sulfoximine-induced cataract formation in newborn rats.
SO FREE RADICAL BIOLOGY AND MEDICINE, **(1995 Apr)** 18 (4) 823-9.
Journal code: FRE; 8709159. ISSN: 0891-5849.
AB We investigated the effect of **alpha-lipoic acid**, a powerful antioxidant, on cataract formation in L-buthionine(S,R)-sulfoximine (BSO)-treated newborn rats and found that a dose of 25 mg/kg b.w.. . . therapeutic antioxidants in protecting animals from cataract formation. Major biochemical changes in the lens associated with the protective effect of **alpha-lipoic acid** were increases in glutathione, ascorbate, and vitamin E levels, loss of which are effects of BSO administration. Treatment with **alpha-lipoic acid** also restored the activities of glutathione peroxidase, catalase, and ascorbate free radical reductase in lenses of L-buthionine(S,R)-sulfoximine-treated animals but did not affect glutathione reductase or superoxide dismutase activity. We conclude that **alpha-lipoic acid** may take over some of the functions of glutathione (e.g., maintaining the higher level of ascorbate, indirect participation in vitamin. . . the increase of glutathione level in lens tissue mediated by lipoate could be also due to a direct protection of **protein** thiols. Thus, **alpha-lipoic acid** could be of potential therapeutic use in preventing cataracts and their complications.
CT Check Tags: Animal; Female; Human; Male; Support, U.S. Gov't, P.H.S.
Buthionine Sulfoximine
*Cataract: CI, chemically induced
Free Radicals
Glutamate-Cysteine Ligase: AI, antagonists & inhibitors
Glutamate-Cysteine Ligase: PD, pharmacology
Glutathione: PD, pharmacology
Infant, Newborn
Lenses
*Methionine Sulfoximine: AA, analogs & derivatives
Methionine Sulfoximine: PD, pharmacology
. . .
CN 0 (Free Radicals); EC 6.3.2.2 (Glutamate-**Cysteine** Ligase)
AN 95270045 MEDLINE
DN 95270045 PubMed ID: 7750805
TI **Alpha-lipoic acid** prevents buthionine sulfoximine-induced cataract formation in newborn rats.
AU Maitra I; Serbinova E; Trischler H; Packer L
CS Department of Molecular and Cell Biology, University of California, Berkeley 94720, USA.
NC CA 47957 (NCI)
SO FREE RADICAL BIOLOGY AND MEDICINE, **(1995 Apr)** 18 (4) 823-9.
Journal code: FRE; 8709159. ISSN: 0891-5849.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199506
ED Entered STN: 19950629
Last Updated on STN: 19980206
Entered Medline: 19950621

L7 ANSWER 23 OF 82 MEDLINE
TI Reversal of fungitoxicity of 8-quinolinols and their copper(II) bischelates. II. Reversal of the action of 8-quinolinol by DL-**alpha-lipoic acid**.
SO CANADIAN JOURNAL OF MICROBIOLOGY, **(1981 Jun)** 27 (6) 612-26.
Journal code: CJ3; 0372707. ISSN: 0008-4166.
AB The effect of **amino acids** and derivatives, Krebs cycle acids and related compounds, fatty acids, and vitamins and related compounds on the toxicity of 8-quinolinol and bis(8-quinolinolato)copper(II) to Aspergillus oryzae (ATCC 1011) was studied. Only aliphatic thiol-containing compounds (**cysteine**,

glutathione, dithioerythritol, and dithiothreitol) and DL-**alpha-lipoic acid** protected against 8-quinolinol but not its copper(II) bischelate. It is suggested that 8-quinolinol inhibits lipoic acid biosynthesis, and the mode. . .

CT Check Tags: Support, Non-U.S. Gov't
Amino Acids: PD, pharmacology
*Aspergillus: DE, drug effects
Chelating Agents
Citric Acid Cycle
Copper
Cysteine: PD, pharmacology
Dithiothreitol: PD, pharmacology
Glutathione: PD, pharmacology
*Hydroxyquinolines: AI, antagonists & inhibitors
*Oxyquinoline: AI, antagonists & inhibitors
Oxyquinoline:. . .

RN 148-24-3 (Oxyquinoline); 3483-12-3 (Dithiothreitol); **52-90-4 (Cysteine)**; 62-46-4 (Thioctic Acid); 70-18-8 (Glutathione); 7440-50-8 (Copper)

CN 0 (**Amino Acids**); 0 (Chelating Agents); 0 (Hydroxyquinolines); 0 (Vitamins)

AN 81258086 MEDLINE

DN 81258086 PubMed ID: 6790147

TI Reversal of fungitoxicity of 8-quinolinols and their copper(II) bischelates. II. Reversal of the action of 8-quinolinol by DL-**alpha-lipoic acid**.

AU Gershon H; Shanks L

SO CANADIAN JOURNAL OF MICROBIOLOGY, **(1981 Jun)** 27 (6) 612-26.
Journal code: CJ3; 0372707. ISSN: 0008-4166.

CY Canada

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 198110

ED Entered STN: 19900316
Last Updated on STN: 20000303
Entered Medline: 19811025

L7 ANSWER 24 OF 82 CAPLUS COPYRIGHT 2001 ACS

SO Free Radical Biol. Med. (**1998**), Volume Date 1999, 26(1/2), 174-183
CODEN: FRBMEH; ISSN: 0891-5849

AB . . . signalling pathways leading to gene expression has not been clearly established. In the present study, the effects of the antioxidants .**alpha.-lipoic acid**, N-acetyl-L-**cysteine** (NAC) and the flavonoid ext. silymarin were investigated for their ability to modulate the activation of the transcription factors nuclear factor kappa B (NF-.kappa.B) and activator **protein**-1 (AP-1) in HaCaT keratinocytes after exposure to a solar UV simulator. The activation of NF-.kappa.B and AP-1 showed a similar.

. were evaluated 3 h after exposure. While a high concn. of NAC could achieve a complete inhibition, low concns. of .**alpha.-lipoic acid** and silymarin were shown to significantly inhibit NF-.kappa.B activation. In contrast, AP-1 activation was only partially inhibited by NAC, and not at all by .**alpha.-lipoic acid** or silymarin. These results indicate that antioxidants such as .**alpha.-lipoic acid** and silymarin can efficiently modulate the cellular response to UVR through their selective action on NF-.kappa.B activation.

IT 616-91-1, N-Acetyl-L-**cysteine** 1200-22-2, .**alpha.-Lipoic acid** 65666-07-1, Silymarin
RL: BAC (Biological activity or effector, except adverse); BIOL (Biological study)
(antioxidants modulate acute solar UV radiation-induced NF-kappa-B activation in a human keratinocyte cell line)

AN 1999:1589 CAPLUS
DN 130:164960
TI Antioxidants modulate acute solar ultraviolet radiation-induced NF-kappa-B
activation in a human keratinocyte cell line
AU Saliou, Claude; Kitazawa, Manabu; McLaughlin, Laura; Yang, Jian-Ping; Lodge, John K.; Tetsuka, Toshifumi; Iwasaki, Keiji; Cillard, Josiane; Okamoto, Takashi; Packer, Lester
CS Department of Molecular and Cell Biology, University of California, Berkeley, CA, 94720-3200, USA
SO Free Radical Biol. Med. (**1998**), Volume Date 1999, 26(1/2), 174-183
CODEN: FRBMEH; ISSN: 0891-5849
PB Elsevier Science Inc.
DT Journal
LA English
RE.CNT 55
RE
(1) Agarwal, R; Photochem Photobiol 1996, V63, P440 CAPLUS
(4) Baldwin, A; Annu Rev Immunol 1996, V14, P649 CAPLUS
(5) Beg, A; Genes Dev 1995, V9, P2736 CAPLUS
(6) Beissert, S; Crit Rev Biochem Mol Biol 1996, V31, P381 CAPLUS
(7) Bindoli, A; Biochem Pharmacol 1977, V26, P2405 CAPLUS
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L7 ANSWER 25 OF 82 CAPLUS COPYRIGHT 2001 ACS
PI WO 9843621 A1 **19981008**

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9843621	A1	19981008	WO 1998-US6287	19980331 <--
W: CA, JP				
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
EP 979073	A1	20000216	EP 1998-913316	19980331
R: DE, ES, FR, GB, IT				

AB S-nitrosylation (reaction of nitric oxide [NO] species with crit. **cysteine** sulfhydryl groups of a caspase [RS] to form RS-NO) inhibits caspase activity and thereby ameliorates apoptosis not only in neuronal. . .
IT **Proteins** (specific **proteins** and subclasses)
RL: BAC (Biological activity or effector, except adverse); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(antennapedia, conjugates with pseudocaspase enzymes; nitrosylation to inactivate apoptotic enzymes, and therapeutic caspase-like peptide)
IT 50-81-7, L-**Ascorbic acid**, biological studies
55-63-0, Nitroglycerin 70-18-8, Glutathione, biological studies
87-33-2, Isosorbide dinitrate 281-23-2D, Adamantane, derivs., NO reaction products 462-20-4 599-71-3, Piloty's acid 1200-22-2, **.alpha.-Lipoic acid** 1406-18-4, Vitamin E
7439-89-6D, Iron, SIN-1-nitrosyl complexes 9004-08-4D, Cathepsin, NO reaction products 13826-64-7, Angeli's salt 14402-89-2, Sodium nitroprusside 19982-08-2D, Memantine, NO reaction products
33876-97-0D, SIN-1, cation-nitrosyl complexes 51209-75-7, S-Nitrosocysteine 57564-91-7, S-Nitrosoglutathione 61142-90-3
65141-46-0, Nicorandil 72909-34-3, Pyrroloquinoline quinone
72909-34-3D, Pyrroloquinoline quinone, ester derivs. 122130-63-6, S-Nitrosocaptopril 139639-23-9D, Tissue plasminogen activator, NO reaction products 197771-66-7 197771-67-8
RL: BAC (Biological activity or effector, except adverse); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(nitrosylation to inactivate apoptotic enzymes, and therapeutic caspase-like peptide)
AN 1998:682105 CAPLUS
DN 129:298408
TI Nitrosylation to inactivate apoptotic enzymes, and therapeutic caspase-like peptide

IN Lipton, Stuart A.; Troy, Carol M.
PA The Children's Medical Center Corp., USA
SO PCT Int. Appl., 20 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE	
PI	WO 9843621	A1	19981008	WO 1998-US6287	19980331	<--
	W: CA, JP					
	RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE					
	EP 979073	A1	20000216	EP 1998-913316	19980331	
	R: DE, ES, FR, GB, IT					
PRAI	US 1997-42144		19970331			
	WO 1998-US6287		19980331			

OS MARPAT 129:298408

L7 ANSWER 26 OF 82 CAPLUS COPYRIGHT 2001 ACS
PI EP 869126 A1 **19981007**

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE	
PI	EP 869126	A1	19981007	EP 1998-302532	19980401	<--
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO					
	AU 9859702	A1	19981008	AU 1998-59702	19980330	<--
	AU 728488	B2	20010111			
	US 6013663	A	20000111	US 1998-52095	19980331	
	ZA 9802766	A	19980930	ZA 1998-2766	19980401	<--
	CA 2233682	AA	19981002	CA 1998-2233682	19980401	<--
	NO 9801469	A	19981005	NO 1998-1469	19980401	<--
	JP 11269170	A2	19991005	JP 1998-89033	19980401	
	EP 1070710	A2	20010124	EP 2000-122586	19980401	
	EP 1070710	A3	20010321			
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI					
	CN 1208035	A	19990217	CN 1998-102916	19980402	

AB . . . be used for treatment and prevention of a variety of diseases, esp. cataracts. Over 125 examples are given. For instance, D,L-.**alpha.-lipoic acid** was activated with N,N'-carbonyldiimidazole in DMF and then treated with MeSO2NH2 and NaH to give title compd. II. This compd.. . .

IT 56-92-8, Histamine dihydrochloride 57-14-7, 1,1-Dimethylhydrazine 67-56-1, Methanol, reactions 74-89-5, Methylamine, reactions 85-41-6, Phthalimide 96-32-2, Methyl bromoacetate 98-10-2, Benzenesulfonamide 98-88-4, Benzoyl chloride 107-18-6, 2-Propen-1-ol, reactions 107-35-7, 2-Aminoethanesulfonic acid 108-30-5, reactions 108-55-4, Glutaric anhydride 109-73-9, Butylamine, reactions 110-85-0, Piperazine, reactions 110-89-4, Piperidine, reactions 110-91-8, Morpholine, reactions 123-62-6, Propionic anhydride 123-75-1, Pyrrolidine, reactions 123-90-0, Thiomorpholine 124-40-3, Dimethylamine, reactions 141-43-5, reactions 504-78-9, Thiazolidine 530-62-1, N,N'-Carbonyldiimidazole 541-41-3, Ethyl chloroformate 556-61-6, Methyl isothiocyanate 608-07-1, 5-Methoxytryptamine 940-69-2, 1,2-Dithiolane-3-pentanamide 1077-28-7, D,L-.**alpha.-Lipoic acid** 1118-02-1, Trimethylsilyl isocyanate 1200-22-2, (R)-.**alpha.-Lipoic acid** 1520-70-3, Ethanesulfonamide 1668-10-6, Glycinamide hydrochloride 2213-43-6, 1-Aminopiperidine 2491-20-5, L-**Alanine** methyl ester hydrochloride 3144-09-0, Methanesulfonamide 3196-73-4, .beta.-**Alanine** methyl ester hydrochloride 3395-91-3, Methyl 3-bromopropionate 3518-65-8, Chloromethanesulfonyl chloride 5680-79-5, Glycine methyl ester hydrochloride 5781-53-3, Methyloxalyl chloride

6168-72-5, D,L-Alaninol 7389-87-9, L-Histidine methyl ester dihydrochloride 7803-58-9, Sulfamide 13031-60-2, Methyl 4-aminobutanoate hydrochloride 14316-06-4, D-**Alanine** methyl ester hydrochloride 20045-77-6, N-Methyl-L-**alanine** methyl ester hydrochloride 20260-53-1, Nicotinoyl chloride hydrochloride 29840-56-0, Methyl 5-aminopentanoate hydrochloride 52605-49-9, Sarcosine ethyl ester hydrochloride 57260-71-6, N-(tert-Butoxycarbonyl)piperazine 59040-84-5, Methyl indoline-2-carboxylate 61314-87-2, S-(4-Methoxybenzyl)-L-**cysteine** methyl ester 79475-92-6, 4,6-Dithioxyhexanoic acid 89584-24-7, Methyl 4-(methylamino)butanoate hydrochloride 99663-32-8 127254-35-7, (S)-.**alpha**.-**Lipoic acid** 148556-90-5, 2-Nitroxyethylamine hydrochloride 174724-41-5 186376-29-4, 1-Methyl-2-nitroxyethylamine hydrochloride 198016-53-4, Methyl indoline-2-carboxylate hydrochloride 214556-14-6 214556-16-8 214556-18-0
RL: RCT (Reactant)
(starting material; prepn. of dithiolan derivs. as glutathione reductase enhancers)

AN 1998:668121 CAPLUS
DN 129:302641
TI Dithiolan derivatives, their preparation, and their therapeutic effect as glutathione reductase enhancers
IN Fujita, Takashi; Yokoyama, Tomihisa
PA Sankyo Co., Ltd., Japan
SO Eur. Pat. Appl., 415 pp.
CODEN: EPXXDW
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE	
PI	EP 869126	A1	19981007	EP 1998-302532	19980401	<--
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO					
	AU 9859702	A1	19981008	AU 1998-59702	19980330	<--
	AU 728488	B2	20010111			
	US 6013663	A	20000111	US 1998-52095	19980331	
	ZA 9802766	A	19980930	ZA 1998-2766	19980401	<--
	CA 2233682	AA	19981002	CA 1998-2233682	19980401	<--
	NO 9801469	A	19981005	NO 1998-1469	19980401	<--
	JP 11269170	A2	19991005	JP 1998-89033	19980401	
	EP 1070710	A2	20010124	EP 2000-122586	19980401	
	EP 1070710	A3	20010321			
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI					
	CN 1208035	A	19990217	CN 1998-102916	19980402	
PRAI	JP 1997-83749	A	19970402			
	JP 1998-8837	A	19980120			
	EP 1998-302532	A3	19980401			

OS MARPAT 129:302641

L7 ANSWER 27 OF 82 CAPLUS COPYRIGHT 2001 ACS
SO Free Radical Biol. Med. (**1998**), 25(2), 229-241
CODEN: FRBMEH; ISSN: 0891-5849
AB . . . (50-250 .mu.M). Inhibition of PMA-induced adhesion mol. expression and cell-cell adhesion was synergized when a combination of antioxidants, .alpha.-lipoate and .alpha.-**tocopherol**, was used compared to the use of either of these antioxidants alone. The regulation of adhesion mol. expression and function. . . not appear to be nuclear factor .kappa.B regulated or transcription dependent, because no change in the mRNA response was obsd. **Protein** kinase C has been suggested to regulate PMA-induced adhesion mol. expression by post-transcriptional stabilization of adhesion mol. mRNA; however, .alpha.-lipoate pretreatment

did not influence the response of **protein** kinase C activity to PMA. Oxidants are known to be involved in the regulation of cell adhesion processes. Treatment of. . . treatment decreased PMA-induced generation of intracellular oxidants. The inhibitory effect of low concn. of .alpha.-lipoate alone or in combination with .alpha.-**tocopherol** on agonist-induced adhesion processes obsd. in this study may be of potential therapeutic value.

IT 59-02-9, .alpha.-**Tocopherol** 950-99-2, 2,2,5,7,8-Pentamethyl-6-hydroxychroman 1200-22-2, .**alpha**.-**Lipoic acid** 16561-29-8, Phorbol myristate acetate
RL: BAC (Biological activity or effector, except adverse); BIOL (Biological study)
(antioxidant regulation of phorbol ester-induced adhesion of human Jurkat T-cells to endothelial cells)

IT 141436-78-4, **Protein** kinase C
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(antioxidant regulation of phorbol ester-induced adhesion of human Jurkat T-cells to endothelial cells)

AN 1998:439899 CAPLUS
DN 129:173629
TI Antioxidant regulation of phorbol ester-induced adhesion of human Jurkat T-cells to endothelial cells
AU Roy, Sashwati; Sen, Chandan K.; Kobuchi, Hirotsugu; Packer, Lester
CS Membrane Bioenergetics Group, Department of Molecular and Cell Biology, University of California, Berkeley, CA, 94720-3200, USA
SO Free Radical Biol. Med. (**1998**), 25(2), 229-241
CODEN: FRBMEH; ISSN: 0891-5849
PB Elsevier Science Inc.
DT Journal
LA English

L7 ANSWER 28 OF 82 CAPLUS COPYRIGHT 2001 ACS
SO Biochem. Pharmacol. (**1998**), 55(11), 1747-1758
CODEN: BCPCA6; ISSN: 0006-2952
AB . . . a fundamental regulatory mechanism in cell biol. Electron flow through side chain functional CH2-SH groups of conserved cysteinyl residues in **proteins** account for their redox-sensing properties. Because in most intracellular **proteins** thiol groups are strongly "buffered" against oxidn. by the highly reduced environment inside the cell, only accessible **protein** thiol groups with high thiol-disulfide oxidn. potentials are likely to be redox sensitive. The list of redox-sensitive signal transduction pathways. . . are of central importance in redox signaling. Among the thiol agents tested for their efficacy to modulate cellular redox status, N-acetyl-L-**cysteine** (NAC) and .**alpha**.-**lipoic acid** hold promise for clin. use. A unique advantage of lipoate is that it is able to utilize cellular reducing equiv.,. . . regenerate its reductive vicinal dithiol form. Because lipoate can be readily recycled in the cell, it has an advantage over N-acetyl-L-**cysteine** on a concn./effect basis. Our current knowledge of redox regulated signal transduction has led to the unfolding of the remarkable. . .

AN 1998:383234 CAPLUS
DN 129:117304
TI Redox signaling and the emerging therapeutic potential of thiol antioxidants
AU Sen, Chandan K.
CS Department of Molecular and Cell Biology, University of California, Berkeley, CA, 94720-3200, USA
SO Biochem. Pharmacol. (**1998**), 55(11), 1747-1758
CODEN: BCPCA6; ISSN: 0006-2952
PB Elsevier Science Inc.
DT Journal; General Review
LA English

L7 ANSWER 29 OF 82 CAPLUS COPYRIGHT 2001 ACS
TI .alpha.-**Lipoic acid** in liver metabolism and disease
SO Free Radical Biol. Med. (**1998**), 24(6), 1023-1039
CODEN: FRBMEH; ISSN: 0891-5849
AB A review with 141 refs. R-.alpha.-**Lipoic acid** is found naturally occurring as a prosthetic group in .alpha.-keto acid dehydrogenase complexes of the mitochondria, and as such plays a fundamental role in metab. Although this has been known for decades, only recently has free supplemented .alpha.-**lipoic acid** been found to affect cellular metabolic processes in vitro, as it has the ability to alter the redox status of. . . it appears that this compd. has important therapeutic potential in conditions where oxidative stress is involved. Early case studies with .alpha.-**lipoic acid** were performed with little knowledge of the action of .alpha.-**lipoic acid** at a cellular level, but with the rationale that because the naturally occurring **protein** bound form of .alpha.-**lipoic acid** has a pivotal role in metab., that supplementation may have some beneficial effect. Such studies sought to evaluate the effect of supplemented .alpha.-**lipoic acid**, using low doses, on lipid or **carbohydrate** metab., but little or no effect was obsd. A common response in these trials was an increase in glucose uptake,. . . lactate were also obsd., suggesting that an inhibitory effect on the pyruvate dehydrogenase complex was occurring. During the same period, .alpha.-**lipoic acid** was also used as a therapeutic agent in a no. of conditions relating to liver disease, including alc.-induced damage, mushroom poisoning, metal intoxification, and CCl4 poisoning. .alpha.-**Lipoic acid** supplementation was successful in the treatment for these conditions in many cases. Exptl. studies and clin. trials in the last 5 yr using high doses of .alpha.-**lipoic acid** (600 mg in humans) have provided new and consistent evidence for the therapeutic role of antioxidant .alpha.-**lipoic acid** in the treatment of insulin resistance and diabetic polyneuropathy. This new insight should encourage clinicians to use .alpha.-**lipoic acid** in diseases affecting liver in which oxidative stress is involved.
IT Antioxidants (pharmaceutical)
Lipid metabolism
Liver
Liver diseases
(.alpha.-**lipoic acid** in human liver metab. and disease)
IT 1200-22-2, .alpha.-**Lipoic acid**
RL: BAC (Biological activity or effector, except adverse); BOC (Biological occurrence); BPR (Biological process); THU (Therapeutic use); BIOL (Biological study); OCCU (Occurrence); PROC (Process); USES (Uses)
(.alpha.-**lipoic acid** in human liver metab. and disease)
AN 1998:301796 CAPLUS
DN 129:35996
TI .alpha.-**Lipoic acid** in liver metabolism and disease
AU Bustamante, Juanita; Lodge, John K.; Marcocci, Lucia; Tritschler, Hans J.; Packer, Lester; Rihn, Bertrand H.
CS Membranes Bioenergetics Group, Department of Molecular and Cell Biology, University of California, Berkeley, CA, USA
SO Free Radical Biol. Med. (**1998**), 24(6), 1023-1039
CODEN: FRBMEH; ISSN: 0891-5849
PB Elsevier Science Inc.

DT Journal; General Review
LA English

L7 ANSWER 30 OF 82 CAPLUS COPYRIGHT 2001 ACS
PI US 5691203 A **19971125**

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI US 5691203	A	19971125	US 1993-128225	19930929 <--
JP 07008273	A2	19950113	JP 1993-141984	19930614 <--

IT 50-23-7, Hydrocortisone 50-89-5, Thymidine, biological studies 50-99-7, D-Glucose, biological studies 52-90-4, **Cysteine**, biological studies 56-40-6, Glycine, biological studies 56-41-7, **Alanine**, biological studies 56-45-1, L-Serine, biological studies 56-84-8, L-Aspartic acid, biological studies 56-85-9, **Glutamine**, biological studies 56-86-0, Glutamic acid, biological studies 56-87-1, Lysine, biological studies 58-05-9, Folinic acid 58-85-5, D-Biotin 59-43-8, Thiamine, biological studies 60-18-4, Tyrosine, biological studies 61-90-5, L-**Leucine**, biological studies 63-68-3, Methionine, biological studies 63-91-2, **Phenylalanine**, biological studies 65-23-6, Pyridoxine 67-48-1 68-19-9, Vitamin B12 70-47-3, Asparagine, biological studies 71-00-1, Histidine, biological studies 72-18-4, **Valine**, biological studies 72-19-5, Threonine, biological studies 73-22-3, Tryptophan, biological studies 73-24-5, Adenine, biological studies 73-32-5, **Isoleucine**, biological studies 74-79-3, L-**Arginine**, biological studies 79-83-4, D-Pantothenic acid 83-88-5, Riboflavin, biological studies 87-67-2, Choline bitartrate, biological studies 87-89-8, myo-**Inositol** 98-92-0, Nicotinamide 110-60-1, Putrescine 113-24-6, Sodium pyruvate 137-08-6 143-74-8, Phenol red 144-55-8, Carbonic acid monosodium salt, biological studies 147-85-3, Proline, biological studies 1200-22-2, .**alpha.-Lipoic acid** 1344-09-8 1492-18-8, Calcium folinate 7447-40-7, Potassium chloride (KCl), biological studies 7487-88-9, Sulfuric acid magnesium salt (1:1), biological studies 7558-79-4 7647-14-5, Sodium chloride, biological studies 7718-54-9, Nickel chloride (NiCl2), biological studies 7720-78-7, Sulfuric acid iron(2+) salt (1:1) 7733-02-0 7758-98-7, Sulfuric acid copper(2+) salt (1:1), biological studies 7772-99-8, Tin chloride (SnCl2), biological studies 7778-77-0 7785-87-7 7803-55-6 10043-52-4, Calcium chloride (CaCl2), biological studies 10102-18-8 12027-67-7 91037-65-9 93674-99-8 110590-64-2 129058-83-9 134580-64-6
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(method for serum-free culture of human vascular endothelial cells)

AN 1997:761704 CAPLUS
DN 128:45595
TI Method for serum-free culture of human vascular endothelial cells
IN Katsuen, Susumu; Ohshima, Kunihiro; Yamamoto, Ryohei; Nishino, Toyokazu
PA Kurashiki Boseki Kabushiki Kaisha, Japan
SO U.S., 11 pp.
CODEN: USXXAM
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI US 5691203	A	19971125	US 1993-128225	19930929 <--
JP 07008273	A2	19950113	JP 1993-141984	19930614 <--
PRAI JP 1993-141984		19930614		

L7 ANSWER 31 OF 82 CAPLUS COPYRIGHT 2001 ACS
SO Biochem. Mol. Biol. Int. (**1997**), 42(6), 1189-1197
CODEN: BMBIES; ISSN: 1039-9712
AB The effect of several antioxidants and **cysteine**-elevating precursor drugs (prodrugs) was tested on lens damage occurring after in vitro exposure to low levels of 60Co-.gamma.-irradn., to simulate in vitro

the exposure to radiation in vivo of (1) astronauts (2) jet crews (3) military radiation accident personnel. **Tocopherol** (100 .mu.M), **ascorbic acid** (1mM), R-.**alpha.-lipoic acid** (1mM), and taurine (0.5 mM) protected against radiation-assocd. **protein** leakage. MTCA and ribocysteine protected lenses against opacification, LDH and **protein** leakage, indicating that antioxidants and prodrugs of **cysteine** appear to offer protection against lens damage caused by low level radiation.

IT **Tocopherols**
RL: BAC (Biological activity or effector, except adverse); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(antioxidants treatment of 60Co-.gamma.-irradn.-induced cataracts)

IT 50-81-7, **Ascorbic acid**, biological studies 107-35-7, Taurine 1200-22-2, R-.**alpha.-Lipoic acid** 17087-36-4 190062-99-8
RL: BAC (Biological activity or effector, except adverse); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(antioxidants treatment of 60Co-.gamma.-irradn.-induced cataracts)

AN 1997:719001 CAPLUS
DN 128:57401
TI Antioxidants and cataract: (cataract induction in space environment and application to terrestrial aging cataract)
AU Bantseev, Vladimir; Bhardwaj, Ratan; Rathbun, W.; Nagasawa, H.; Trevithick, John R.
CS Department of Biochemistry, University of Western Ontario, London, ON, N6A 5C1, Can.
SO Biochem. Mol. Biol. Int. (**1997**), 42(6), 1189-1197
CODEN: BMBIES; ISSN: 1039-9712
PB Academic
DT Journal
LA English

L7 ANSWER 32 OF 82 CAPLUS COPYRIGHT 2001 ACS
TI Advanced glycation end product-induced activation of NF-.kappa.B is suppressed by .**alpha.-lipoic acid** in cultured endothelial cells
SO Diabetes (**1997**), 46(9), 1481-1490
CODEN: DIAEAZ; ISSN: 0012-1797
AB . . . cultured bovine aortic endothelial cells (BAECs) with AGE albumin (500 nmol/l) resulted in the impairment of reduced glutathione (GSH) and **ascorbic acid** levels. As a consequence, increased cellular oxidative stress led to the activation of the transcription factor NF-.kappa.B and thus promoted. . . upregulation of various NF-.kappa.B-controlled genes, including endothelial tissue factor. Supplementation of the cellular antioxidative defense with the natural occurring antioxidant .**alpha.-lipoic acid** before AGE albumin induction completely prevented the AGE albumin-dependent depletion of reduced glutathione and **ascorbic acid**. Electrophoretic mobility shift assays (EMSAs) revealed that AGE albumin-mediated NF-.kappa.B activation was also reduced in a time- and dose-dependent manner as long as .**alpha.-lipoic acid** was added at least 30 min before AGE albumin stimulation. Inhibition was not due to phys. interactions with **protein** DNA binding, since .**alpha.-lipoic acid**, directly included into the binding reaction, did not prevent binding activity of recombinant NF-.kappa.B. Western blots further demonstrated that .**alpha.-lipoic acid** inhibited the release and translocation of NF-.kappa.B from the cytoplasm into the nucleus. As a consequence, .**alpha.-lipoic acid** reduced AGE albumin-induced NF-.kappa.B mediated transcription and expression of endothelial genes relevant in diabetes, such as tissue factor and endothelin-1. Thus, supplementation of cellular antioxidative defense mechanisms by extracellularly administered .**alpha.-lipoic acid** reduces AGE albumin-induced endothelial

dysfunction in vitro.
IT Genes (animal)
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(for tissue factor and endothelin-1; .**alpha.-lipoic acid** suppression of advanced glycation end product-induced activation of NF-.kappa.B in vascular endothelium)
IT Antioxidants
Diabetic angiopathy
Vascular endothelium
(.**alpha.-lipoic acid** suppression of advanced glycation end product-induced activation of NF-.kappa.B in vascular endothelium)
IT NF-.kappa.B
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(.**alpha.-lipoic acid** suppression of advanced glycation end product-induced activation of NF-.kappa.B in vascular endothelium)
IT Advanced glycation end products
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(.**alpha.-lipoic acid** suppression of advanced glycation end product-induced activation of NF-.kappa.B in vascular endothelium)
IT 1200-22-2, .**alpha.-Lipoic acid**
RL: BAC (Biological activity or effector, except adverse); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(.**alpha.-lipoic acid** suppression of advanced glycation end product-induced activation of NF-.kappa.B in vascular endothelium)
IT 9035-58-9, Blood-coagulation factor III 123626-67-5, Endothelin-1
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(.**alpha.-lipoic acid** suppression of advanced glycation end product-induced activation of NF-.kappa.B in vascular endothelium)
AN 1997:593495 CAPLUS
DN 127:272756
TI Advanced glycation end product-induced activation of NF-.kappa.B is suppressed by .**alpha.-lipoic acid** in cultured endothelial cells
AU Bierhaus, Angelika; Chevion, Shlomit; Chevion, Mordechai; Hofmann, Marion;
Quehenberger, Peter; Illmer, Thomas; Luther, Thomas; Berentshtein, Eduard;
Tritschler, Hans; Muller, Martin; Wahl, Peter; Ziegler, Reinhard; Nawroth,
Peter P.
CS Department of Internal Medicine, University of Heidelberg, Heidelberg, Germany
SO Diabetes (**1997**), 46(9), 1481-1490
CODEN: DIAEAZ; ISSN: 0012-1797
PB American Diabetes Association, Inc.
DT Journal
LA English

L7 ANSWER 33 OF 82 CAPLUS COPYRIGHT 2001 ACS
TI Regulation of cellular thiols in human lymphocytes by .**alpha.-lipoic acid**: a flow cytometric analysis
SO Free Radical Biol. Med. (**1997**), 22(7), 1241-1257
CODEN: FRBMEH; ISSN: 0891-5849
AB . . . its fatty acid structure. In certain diseases such as AIDS and cancer, elevated plasma glutamate lowers cellular GSH by inhibiting **cysteine** uptake. Low concns. of lipoate and lipoamide were able to bypass the adverse effect of elevated extracellular glutamate. A heterogeneity. . .
ST lipoate glutathione **glutamine** thiol lymphocyte AIDS
IT JURKAT cell
(regulation of cellular thiols in Jurkat cell by .**alpha.-**

lipoic acid)

IT Thiols (organic), biological studies
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(regulation of cellular thiols in Jurkat cell by **.alpha.-lipoic acid**)

IT Lymphocyte
(regulation of cellular thiols in human lymphocytes by **.alpha.-lipoic acid**)

IT 56-85-9, **Glutamine**, biological studies
RL: BAC (Biological activity or effector, except adverse); BIOL (Biological study)
(effect of lipoate and lipoamide on cellular glutathione with high concn. of extracellular **glutamine**)

IT 940-69-2, Lipoamide 1200-22-2, **.alpha.-Lipoic acid**
RL: BAC (Biological activity or effector, except adverse); BIOL (Biological study)
(regulation of cellular thiols in human lymphocytes by **.alpha.-lipoic acid**)

IT 70-18-8, GSH, biological studies
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(regulation of cellular thiols in human lymphocytes by **.alpha.-lipoic acid**)

AN 1997:258326 CAPLUS
DN 126:341496
TI Regulation of cellular thiols in human lymphocytes by **.alpha.-lipoic acid**: a flow cytometric analysis
AU Sen, Chandan K.; Roy, Sashwati; Han, Derick; Packer, Lester
CS Dep. Mol. Cell Biol., Univ. California, Berkeley, CA, 94720-3200, USA
SO Free Radical Biol. Med. (**1997**), 22(7), 1241-1257
CODEN: FRBMEH; ISSN: 0891-5849
PB Elsevier
DT Journal
LA English

L7 ANSWER 34 OF 82 CAPLUS COPYRIGHT 2001 ACS
TI Identification of pro-oxidant or antioxidant characteristics of **proteins** and enzymes in membranes; use of liposome-entrapped **proteins** and other thiol-containing compounds
SO Biochem. Soc. Trans. (**1996**), 24(3), 375S
CODEN: BCSTB5; ISSN: 0300-5127
AB . . . their ability to inhibit lipid peroxidn. in multilamellar liposomes prepd. from ox-brain phospholipids. Peroxidn. was induced with iron(III) chloride and **ascorbic acid** and terminated by addn. of BHT. Insulin, homocysteine, and cytochrome c all displayed membrane structural pro-oxidant action when added either. . . exhibit pro-oxidant action. Superoxide dismutase and catalase both showed antioxidant action, esp. when entrapped inside the liposome. Another thiol-contg. compd., **.alpha.-lipoic acid**, showed pro-oxidant effect when added after liposome formation, but showed a significant antioxidant effect when added before liposome formation.
ST lipid peroxidn **protein** enzyme thiol compd; prooxidant antioxidant **protein** enzyme thiol compd
IT Peroxidation
(lipid; pro-oxidant or antioxidant characteristics of **proteins**, enzymes, and thiol-contg. compds. in membranes)
IT Phospholipids, biological studies
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(peroxidn.; pro-oxidant or antioxidant characteristics of **proteins**, enzymes, and thiol-contg. compds. in membranes)
IT Antioxidants
(pro-oxidant or antioxidant characteristics of **proteins**, enzymes, and thiol-contg. compds. in membranes)
IT Thiols, biological studies
RL: ADV (Adverse effect, including toxicity); BAC (Biological activity or effector, except adverse); BIOL (Biological study)

(pro-oxidant or antioxidant characteristics of **proteins**, enzymes, and thiol-contg. compds. in membranes)

IT Oxidizing agents
(pro-oxidants; pro-oxidant or antioxidant characteristics of **proteins**, enzymes, and thiol-contg. compds. in membranes)

IT 454-28-4, Homocysteine 1200-22-2, **.alpha.-Lipoic acid** 9004-10-8, Insulin, biological studies 9007-43-6, Cytochrome c, biological studies 9031-72-5, Alcohol dehydrogenase
RL: ADV (Adverse effect, including toxicity); BAC (Biological activity or effector, except adverse); BIOL (Biological study)
(pro-oxidant or antioxidant characteristics of **proteins**, enzymes, and thiol-contg. compds. in membranes)

IT 9001-05-2, Catalase 9054-89-1, Superoxide dismutase
RL: BAC (Biological activity or effector, except adverse); BIOL (Biological study)
(pro-oxidant or antioxidant characteristics of **proteins**, enzymes, and thiol-contg. compds. in membranes)

AN 1996:570460 CAPLUS
DN 125:241021
TI Identification of pro-oxidant or antioxidant characteristics of **proteins** and enzymes in membranes; use of liposome-entrapped **proteins** and other thiol-containing compounds
AU Straghan, Esther; Sharma, Geeta; Goldfarb, Peter; Wiseman, Alan
CS Molecular Toxicology Group, University of Surrey, Surrey, GU2 5XH, UK
SO Biochem. Soc. Trans. (**1996**), 24(3), 375S
CODEN: BCSTB5; ISSN: 0300-5127
DT Journal
LA English

L7 ANSWER 35 OF 82 CAPLUS COPYRIGHT 2001 ACS
TI Effect of DL **.alpha.-lipoic acid** on tissue redox state in acute cadmium-challenged tissues
SO J. Nutr. Biochem. (**1996**), 7(2), 85-92
CODEN: JNBIEL; ISSN: 0955-2863
AB . . . contributing to the thiol pool of the cell. The present study was designed to det. whether dietary supplementation of DL **.alpha.-lipoic acid** (15 and 30 mg/kg), a "meta-vitamin," to cadmium-intoxicated rats (3 mg/kg) affords protection against the oxidative stress caused by the. . . rats showed elevated levels of hydroxyl radicals and malondialdehyde (basal and induced), a decreased level of antioxidants-reduced glutathione, total thiols, **protein** thiols, nonprotein thiols, ascorbate, .alpha.-**tocopherol** and retinol and antioxidizing enzymes-superoxide dismutase, catalase, .tau.-glutamyl transpeptidase, glutathione peroxidase, glucose-6-phosphate dehydrogenase, glutathione reductase, and glutathione-S-transferase. Lipoate supplementation changed. . . indirectly by bolstering the antioxidants and antioxidizing enzyme defenses. In vitro studies revealed that, among the mono and dithiols (glutathione, **cysteine**, dithiothreitol, and lipoic acid), lipoic acid was the most potent scavenger of free radicals produced during cadmium-induced hepatotoxicity.
The drug. . .

IT Kidney
Liver
Oxidative stress, biological
(effect of DL **.alpha.-lipoic acid** on tissue redox state in acute cadmium-challenged tissues)

IT Radicals, processes
RL: REM (Removal or disposal); PROC (Process)
(scavengers for; effect of DL **.alpha.-lipoic acid** on tissue redox state in acute cadmium-challenged tissues)

IT Electric activity
(potential, redox, effect of DL **.alpha.-lipoic acid** on tissue redox state in acute cadmium-challenged tissues)

IT 7440-43-9, Cadmium, biological studies
RL: ADV (Adverse effect, including toxicity); BPR (Biological process); BIOL (Biological study); PROC (Process)
(effect of DL **.alpha.-lipoic acid** on tissue redox state in acute cadmium-challenged tissues)
IT 1077-28-7, DL-**.alpha.-Lipoic acid**
RL: BAC (Biological activity or effector, except adverse); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(effect of DL **.alpha.-lipoic acid** on tissue redox state in acute cadmium-challenged tissues)
AN 1996:130058 CAPLUS
DN 124:200875
TI Effect of DL **.alpha.-lipoic acid** on tissue redox state in acute cadmium-challenged tissues
AU Sumathi, Ramachandran; Baskaran, Govindarajan; Varalakshmi, Palaninathan
CS Department Medical Biochemistry, University Madras, Madras, India
SO J. Nutr. Biochem. (**1996**), 7(2), 85-92
CODEN: JNBIEL; ISSN: 0955-2863
DT Journal
LA English

L7 ANSWER 36 OF 82 CAPLUS COPYRIGHT 2001 ACS
PI DE 4419783 A1 **19951207**

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI DE 4419783	A1	19951207	DE 1994-4419783	19940606 <--

AB A hair tonic-and-conditioning shampoo contg. a synergistic combination of (a) **.alpha.-lipoic acid** or a deriv. or analog thereof and (b) a Se salt, a salt of a condensation product of lauric acid with **protein** hydrolyzate, an Na salt of an undecylenic acid condensation product, a sarcoside of palm kernel fatty acids with methyltaurine or triethanolamine, a water-sol. form of vitamin E or vitamin F, **ascorbic acid**, beer ext., chamomile flower ext., or a dye ext. is useful for treatment of (cytostatic-induced) hair loss, hair growth disorders,. . . brittleness, dandruff, and scalp eczema and pyoderma. The effectiveness of the shampoo presumably results from protection of scalp elastin by **.alpha.-lipoic acid**. Thus, a shampoo contained (R)-**.alpha.-lipoic acid** 2.0, Na2SeO3 0.5, Na fatty alc. polyglycol ether sulfate 40.0, coco fatty acid diethanolamide 1.8, NaCl 0.9, Nutrilan L 5.0,. . .
IT **Protein** hydrolyzates
RL: BAC (Biological activity or effector, except adverse); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(condensation products with lauric acid, salts; hair tonic and conditioning shampoo contg. lipoic acid)
IT 50-81-7, **Ascorbic acid**, biological studies 102-71-6D, fatty amides 107-68-6D, Methyltaurine, fatty amides 112-38-9D, Undecylenic acid, condensation products with **protein** hydrolyzates, sodium salts 143-07-7D, Dodecanoic acid, condensation products with **protein** hydrolyzates, salts 462-20-4, Dihydrolipoic acid 462-20-4D, Dihydrolipoic acid, esters 1200-22-2, (R)-**.alpha.-Lipoic acid** 1200-22-2D, (R)-**.alpha.-Lipoic acid**, esters 1406-18-4, Vitamin E 4722-98-9, Texapon MLS 6629-12-5, Tetranorlipoic acid 7782-49-2D, Selenium, salts 9004-82-4, Texapon N 25 10102-18-8, Sodium selenite 13125-44-5, 1,2-Dithiolane-3-propanoic acid 25322-68-3D, ethers with fatty alcs., sulfates 30007-47-7, Bronidox L 83138-08-3, Dehyton K 98441-85-1 98441-85-1D, esters 99427-00-6, **.alpha.-Lipoic acid** methyl ester 119365-69-4 119365-69-4D, esters 127254-35-7, (S)-**.alpha.-Lipoic acid** 127254-35-7D, (S)-**.alpha.-Lipoic**

acid, esters 141489-42-1, Euperlan PK 771 172852-61-8, 1,2-Dithiolane-3-butanesulfonic acid 172852-62-9 172891-50-8, Lamercin 50-80 172891-56-4, Euperlan PK 776 172891-60-0, Texapon EVR 172891-61-1, Texapon MGOR
RL: BAC (Biological activity or effector, except adverse); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(hair tonic and conditioning shampoo contg. lipoic acid)
AN 1996:61385 CAPLUS
DN 124:97245
TI Hair tonic and conditioning shampoo containing lipoic acid
IN Weischer, Carl Heinrich; Ulrich, Heinz; Schindler, Heward
PA Asta Medica AG, Germany
SO Ger. Offen., 6 pp.
CODEN: GWXXBX
DT Patent
LA German
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	DE 4419783	A1	19951207	DE 1994-4419783	19940606 <--

L7 ANSWER 37 OF 82 CAPLUS COPYRIGHT 2001 ACS
TI Effect of .**alpha**.-**lipoic acid** on the peripheral conversion of thyroxine to triiodothyronine and on serum lipid-, **protein**- and glucose levels
SO Arzneim.-Forsch. (**1991**), 41(12), 1294-8
CODEN: ARZNAD; ISSN: 0004-4172
AB The influence of .**alpha**.-**lipoic acid** (LA) on thyroid hormone metab. and serum lipids and **proteins**, and on glucose levels was investigated in rats. Administration of LA together with T4 for 9 days suppressed the T4. . . LA decreased the triglyceride level by 45%; the decrease induced by T4 or LA plus T4 was not significant. Total **protein** and albumin levels were decreased by LA plus T4 treatment when compared to the LA control. The slight increase in. . . 30%, and LA plus T4 further reduced it by 47%. The triglycerides were not affected. A moderate decrease in total **protein** was obsd. after treatment with T4 plus LA; T4 and LA plus T4 decreased the albumin level. The decrease in. . . T3 from T4 when it is coadministered with T4. LA with T4 exerts a lipid-lowering effect with minimal effects on **protein** and **carbohydrate** metab.
ST lipoate thyroid hormone lipid; thyroxine lipoate lipid metab; triiodothyronine lipoate lipid metab; **protein** serum lipoate thyroxine; glucose serum lipoate thyroxine
IT Glycerides, biological studies
Lipids, biological studies
Proteins, biological studies
RL: BIOL (Biological study)
(of blood serum, lipoic acid and thyroxine effect on)
IT 1200-22-2P, .**alpha**.-**Lipoic acid**
RL: PREP (Preparation)
(triiodothyronine formation from thyroxine inhibition by, lipid of blood serum in relation to)
AN 1992:51297 CAPLUS
DN 116:51297
TI Effect of .**alpha**.-**lipoic acid** on the peripheral conversion of thyroxine to triiodothyronine and on serum lipid-, **protein**- and glucose levels
AU Segermann, J.; Hotze, A.; Ulrich, H.; Rao, G. S.
CS Inst. Clin. Biochem., Univ. Bonn, Bonn, W-5300, Germany
SO Arzneim.-Forsch. (**1991**), 41(12), 1294-8
CODEN: ARZNAD; ISSN: 0004-4172
DT Journal

LA English

L7 ANSWER 38 OF 82 CAPLUS COPYRIGHT 2001 ACS
PI DE 3709851 A1 **19881006**

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	DE 3709851	A1	19881006	DE 1987-3709851	19870324 <--
	EP 284549	A2	19880928	EP 1988-730073	19880323 <--
	EP 284549	A3	19890913		
	EP 284549	B1	19920715		
	R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE				
	AT 78175	E	19920815	AT 1988-730073	19880323 <--
	ES 2052766	T3	19940716	ES 1988-730073	19880323 <--
	JP 63255237	A2	19881021	JP 1988-68349	19880324 <--
	US 5160725	A	19921103	US 1991-638134	19910104 <--

IT **Proteins**, specific or class
RL: ANST (Analytical study)
(A, magnetic particles labeled with, for NMR diagnosis)
IT Sulfonamides
RL: ANST (Analytical study)
(alkane, perfluoro, compds., with **phosphorus** acids and carbonic acid, magnetic particles labeled with, for NMR diagnosis)
IT 50-69-1D, Ribose, carboxy and phospho derivs. 50-99-7D, Glucose, carboxy and phospho derivs. 57-48-7D, Fructose, carboxy and phospho derivs. 57-50-1D, carboxy and phospho derivs. 81-25-4, Cholic acid 87-89-8D, **Inositol**, carboxy and phospho derivs. 112-80-1, Oleic acid, uses and miscellaneous 463-40-1, Linolenic acid 470-55-3D, Stachyose, carboxy and phospho derivs. 475-31-0, Glycocholic acid 512-69-6D, Raffinose, carboxy and phospho derivs. 533-67-5D, Deoxyribose, carboxy and phospho derivs. 546-62-3D, Verbascose, carboxy and phospho derivs. 597-12-6D, carboxy and phospho derivs. 1402-10-4D, Lichenin, carboxy and phospho derivs. 9000-69-5, Pectin 9003-01-4, Poly(acrylic acid) 9004-53-9D, Dextrin, carboxy and phospho derivs. 9004-54-0D, Dextran, carboxy and phospho derivs. 9005-25-8D, Starch, carboxy and phospho derivs. 9005-32-7, Alginic acid 9005-79-2D, Glycogen, carboxy and phospho derivs. 9005-80-5D, Inulin, carboxy and phospho derivs. 9011-13-6, Styrene-maleic anhydride copolymer 9013-95-0D, Levan, carboxy and phospho derivs. 9014-63-5D, Xylan, carboxy and phospho derivs. 9036-88-8D, Mannan, carboxy and phospho derivs. 9037-55-2D, Galactan, carboxy and phospho derivs. 9037-90-5D, Fructosan, carboxy and phospho derivs. 9060-75-7D, L-Arabinan, carboxy and phospho derivs. 25087-26-7, Poly(methacrylic acid) 25249-06-3, Polygalacturonic acid 25954-44-3D, carboxy and phospho derivs. 27416-86-0, Polyuridylic acid 29894-36-8, Polymannuronic acid 36655-86-4, Polyglucuronic acid 60-33-3, Linoleic acid, uses and miscellaneous
RL: ANST (Analytical study)
(as stabilizers, for magnetic particles for NMR diagnosis)
IT 58-85-5, Biotin 135-16-0, Tetrahydrofolic acid 462-20-4, Dihydrolipoic acid 576-19-2 1200-22-2, **.alpha.-Lipoic acid** 3432-99-3
RL: ANST (Analytical study)
(in magnetic particle suspension, for NMR diagnosis)
IT 7723-14-0, **Phosphorus**, properties 7782-41-4, Fluorine, properties
RL: PRP (Properties)
(magnetic particles contg., for NMR diagnosis, stabilizing agents for)
AN 1990:51778 CAPLUS
DN 112:51778
TI Stabilized suspension of magnetic particles and its preparation and use in NMR diagnosis
IN Pilgrimm, Herbert

PA Silica Gel G.m.b.H., Fed. Rep. Ger.
SO Ger. Offen., 8 pp.
CODEN: GWXXBX
DT Patent
LA German
FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	DE 3709851	A1	19881006	DE 1987-3709851	19870324 <--
	EP 284549	A2	19880928	EP 1988-730073	19880323 <--
	EP 284549	A3	19890913		
	EP 284549	B1	19920715		
	R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE				
	AT 78175	E	19920815	AT 1988-730073	19880323 <--
	ES 2052766	T3	19940716	ES 1988-730073	19880323 <--
	JP 63255237	A2	19881021	JP 1988-68349	19880324 <--
	US 5160725	A	19921103	US 1991-638134	19910104 <--
PRAI	DE 1987-3709851		19870324		
	EP 1988-730073		19880323		
	US 1988-173590		19880325		

L7 ANSWER 39 OF 82 CAPLUS COPYRIGHT 2001 ACS
TI Reversal of fungitoxicity of 8-quinolinols and their copper(II) bischelates. II. Reversal of the action of 8-quinolinol by DL-**alpha.-lipoic acid**
SO Can. J. Microbiol. (**1981**), 27(6), 612-15
CODEN: CJMIAZ; ISSN: 0008-4166
AB The effects of **amino acids** and derivs., Krebs cycle acids and related compds., fatty acids, and vitamins and related compds. on the toxicity of 8-quinolinol (I) [148-24-3] and Cu bis(8-quinolinolato) [10380-28-6] to Aspergillus oryzae were studied. Only aliph. thiol-contg. compds. (**cysteine**, glutathione, dithioerythritol, and dithiothreitol) and DL-**alpha.-lipoic acid** [1077-28-7] protected against 8-quinolinol, and none of the compds. was effective against the Cu(II) bischelate. 8-Quinolinol may inhibit lipoic acid. . .
IT **Amino acids**, biological studies
Fatty acids, biological studies
Vitamins
RL: BIOL (Biological study)
(fungicidal activity of quinolinols response to)
AN 1981:474483 CAPLUS
DN 95:74483
TI Reversal of fungitoxicity of 8-quinolinols and their copper(II) bischelates. II. Reversal of the action of 8-quinolinol by DL-**alpha.-lipoic acid**
AU Gershon, Herman; Shanks, Larry
CS Boyce Thompson Inst. Plant Res., Cornell Univ., Ithaca, NY, 14853, USA
SO Can. J. Microbiol. (**1981**), 27(6), 612-15
CODEN: CJMIAZ; ISSN: 0008-4166
DT Journal
LA English

L7 ANSWER 40 OF 82 CAPLUS COPYRIGHT 2001 ACS
SO Can. J. Microbiol. (**1972**), 18(7), 1073-8
CODEN: CJMIAZ
AB . . . amts. of acetic and formic acids, and a trace of lactic acid an ethanol. Strain PR-7 required glutamate, aspartate, proline, **leucine**, methionine, **arginine**, **valine**, **alanine**, serine, lysine, glycine, threonine, **isoleucine**, **phenylalanine**, tyrosine, histidine, tryptophan, **glutamine**, asparagine, and spermine. The organism also required nicotinamide, folic acid, pyridoxal, thiamine, riboflavine, pantothenate, choline, . **alpha.-lipoic acid**, and biotin. The fatty acids isobutyrate, n-valerate, acetate, and pyruvate were needed as well as (NH4)2SO4. A fermentable energy source. . . was necessary for

growth, as well as CO2. Heme was also required and the organism was stimulated by vitamin B12, **ascorbic acid**, ornithine, and p-aminobenzoic acid. Heme could be replaced by catalase, myoglobin, and peroxidase. Ten other treponemes that were isolated from. . .

AN 1972:458603 CAPLUS
DN 77:58603
TI Chemically defined medium for Treponema strain PR-7 isolated from the intestine of a pig with swine dysentery
AU Smibert, Robert M.; Claterbaugh, Raymond L., Jr.
CS Anaerobe Lab., Virginia Polytech. Inst., Blacksburg, Va., USA
SO Can. J. Microbiol. (**1972**), 18(7), 1073-8
CODEN: CJMIAZ
DT Journal
LA English

L7 ANSWER 41 OF 82 CAPLUS COPYRIGHT 2001 ACS
TI Stabilizing **.alpha.-lipoic acid** in pharmaceutical solutions
PI DE 1617740 **19711104**

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI DE 1617740	A	19711104	DE 1967-P43060	19670925 <--

AB The light stability of **.alpha.-lipoic acid** in pharmaceutical solns. was increased by the addn. of 70 mg/l. vitamin B6 or a molar equiv. amt. of a. . . KH2PO4 1.36, Na2SO4.10H2O 0.64, NaOAc.3H2O 1.36, Na2CO3 1.64, L-malic acid 2.5, sorbitol 50.0, xylitol 50.0, choline chloride 4.0, methionine 2.0, **arginine** 3.5, glycine 1.0, orotic acid 0.2, inosine 0.05, adenine 0.01, **.alpha.-lipoic acid** 0.20, nicotinic acid amide 0.20, pyridioxine-HCl 0.20, and **inositol** 0.20 g/l. in 0.5 ml EtOH and 500 ml H2O.

AN 1972:17803 CAPLUS
DN 76:17803
TI Stabilizing **.alpha.-lipoic acid** in pharmaceutical solutions
IN Roessler, Richard; Mader, Helmut
PA Pfrimmer, J., und Co.
SO Ger., 4 pp.
CODEN: GWXXAW
DT Patent
LA German
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI DE 1617740	A	19711104	DE 1967-P43060	19670925 <--

L7 ANSWER 42 OF 82 CAPLUS COPYRIGHT 2001 ACS
PI US 3502546 **19700324**

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI US 3502546	A	19700324	US 1966-604570	19661227 <--

AB . . . MgCl2.6H2O 0.177, CaCl2.2H2O 0.0232, dextrose 1.75, Na pyruvate 0.11, l-cystine 0.06, l-glutamic acid 0.063, l-methionine 0.009, l-histidine 0.0168, l-serine 0.021, l-**arginine** 0.632, and l-**glutamine** 0.30 g; FeCl3.6H2O 0.541, ZnSO4.-7H2O 0.863, CoCl2.6H2O 0.048, MnMl2.4H2o 0.099, CuSO4.-5H2O 0.1, riboflavine 0.2, biotin 2, folic acid 2, di-.**alpha.-lipoic acid**, 1, thiamine-HCl 2, chline chloride-2, pyridoxal-HCl 2, cyanocobalamin 0.4, **inositol** 2.16 p-aminobenzoic acid 0.125, niacinamine 2, Ca pantothenate 2, **ascorbic acid** 20, vitamin A alc. 0.25, vitamin D cryst. 0.25, di-.alpha.-**tocopherol** 0.025, vitamin K 2, and phenol red 10 mg; 0.1N H3PO4 7.2 ml. The foregoing is supplemented with 2 g/l. of yeast hydrolyzate contg. various **amino acids** and 5% heat-inactivated rabbit serum. The organism is grown

in either stationary or spinner culture at atm. pressure in a. . .
AN 1970:402067 CAPLUS
DN 73:2067
TI Culture medium and diagnostic method for Treponema pallidum
IN Thompson, Kenneth Wade; Price, Richard Thompson; Prodell, Rita C.; Sipsey,
Matilda M.
PA Organon Inc.
SO U.S., 3 pp.
CODEN: USXXAM
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 3502546	A	19700324	US 1966-604570	19661227 <--

L7 ANSWER 43 OF 82 CAPLUS COPYRIGHT 2001 ACS
TI Lipoic acid derivatives. I. Microbiological activity of DL-.**alpha**.**lipoic acid** derivatives
SO Yakugaku Zasshi (**1965**), 85(5), 460-3
AB Comparative examns. were made on the biol. activity of .**alpha**.-**lipoic acid** (I) and derivs. of I, using Streptococcus faecalis 1 OCI, by the modified Uehara's method (cf. Kamihara, CA 61, 16428h).. . . N-methyl-DL-.alpha.-lipamide and 4-(DL-.alpha.-lipamido)-butyric acid (II) was higher than that of DL-I. II showed almost twice the activity of DL-I. N-DL-.alpha.-Lipoyl-L-tryptophan, N-DL-.alpha.-lipoyl-L-**phenylalanine**, N-DL-.alpha.-lipoyl-L-**leucine**, and N-DL-.alpha.-lipoyl-L-**isoleucine** exhibited lower activity than that of DL-I.
AN 1965:434650 CAPLUS
DN 63:34650
OREF 63:6218b-d
TI Lipoic acid derivatives. I. Microbiological activity of DL-.**alpha**.**lipoic acid** derivatives
AU Okuda, Noriyuki; Fukuda, Yoshio; Oga, Shunichiro; Hirayama, Tadamasa
CS Daiichi Pharm. Co., Tokyo
SO Yakugaku Zasshi (**1965**), 85(5), 460-3
DT Journal
LA Japanese

L7 ANSWER 44 OF 82 CAPLUS COPYRIGHT 2001 ACS
TI The metabolism of .**alpha**.-**lipoic acid** in childhood. I. Effect of **protein** and **amino acid** composition in the diet on the biosynthesis of **protein**-bound lipoic acid
SO J. Vitaminol. (Kyoto) (**1965**), 11(1), 37-44
AB In vitro, the incorporation of labeled lipoic acid into total mitochondrial **protein** fractions from livers of rats maintained on low-**protein** diets was lower than that from rats maintained on standard diets. The incorporation/mg. of N was greater in liver mitochondria from **protein**-deficient animals. Liver mitochondria from rats given intraperitoneal injections of lipoic acid showed similar results. Thus, the levels of lipoic acid activating **proteins** are not easily decreased by low-**protein** diets. Lipoic acid incorporation in vitro and in vivo was decreased in rats fed on a zein diet deficient in tryptophan or lysine. Threonine-deficient and polished-rice diets also depressed the activity of **protein**-bound lipoic acid formation. Methionine- or **phenylalanine**-deficient diets did not affect lipoic acid incorporation.
AN 1965:92966 CAPLUS
DN 62:92966
OREF 62:16681f-g
TI The metabolism of .**alpha**.-**lipoic acid** in childhood. I. Effect of **protein** and **amino acid**

composition in the diet on the biosynthesis of **protein**-bound lipoic acid
AU Nakamura, Tsuneo; Kusunoki, Tomoichi; Konishi, Seizaburo; Kato, Hidehiko; Mibu, Atsuro
CS Prefect. Univ. Med., Kyoto, Japan
SO J. Vitaminol. (Kyoto) (**1965**), 11(1), 37-44
DT Journal
LA English

L7 ANSWER 45 OF 82 CAPLUS COPYRIGHT 2001 ACS
TI Lipoic acid (6,8-dithiooctanoic acid). II. Synthesis of benzhydrylammonium salts of DL-.alpha.-lipoyl-L-**phenylalanine**, -L-methionine and -L-**valine**
SO Zh. Obshch. Khim. (**1964**), 34(11), 3665-7
AB cf. CA 62, 7742f. To DL-.**alpha.-lipoic acid** in Et3N-tetrahydrofuran was added at -5.degree. iso-BuO2CCl in tetrahydrofuran, followed by L-**phenylalanine** in N NaOH; after 0.5 hr. at 20.degree., acidification gave crude DL-.alpha.lipoyl-L-phenyl-**alanine** in the Et2O ext., which with benzhydrylamine gave the corresponding salt, m. 87-8.degree.. Similarly were prepd. benzhydrylammonium DL-.alpha.-lipoyl-L-**valine** salt, m. 108-10.degree., and benzhydrylammonium DL-.alpha.-lipoyl-L-methionine salt, m. 102-4.degree..
AN 1965:44198 CAPLUS
DN 62:44198
OREF 62:7859a-b
TI Lipoic acid (6,8-dithiooctanoic acid). II. Synthesis of benzhydrylammonium salts of DL-.alpha.-lipoyl-L-**phenylalanine**, -L-methionine and -L-**valine**
AU Chebotareva, L. G.; Tursin, V. M.; Luk'yanova, L. V.; Preobrazhenskii, N. A.
CS All-Union Vitamin Res. Inst., Moscow
SO Zh. Obshch. Khim. (**1964**), 34(11), 3665-7
DT Journal
LA Russian

L7 ANSWER 46 OF 82 CAPLUS COPYRIGHT 2001 ACS
TI Model investigations of the chemical carcinogenesis and of the photodynamic effect of 3,4-benzopyrene and ultraviolet light in aqueous **protein** solutions with different sulfhydryl-group reactivities
SO Z. Naturforsch (**1964**), 19b(8), 716-26
AB cf. CA 60, 783e. Fluorimetric detns. showed that 3,4-benzopyrene (I) is much more sol. in aq. **protein** solns. than the non-carcinogenic 1,2-benzopyrene (II). Irradiation of a .beta.-lactoglobulin (III) soln. of I by ultraviolet light of wavelength 366. . . O were the same as caused by irradiation of III alone at 280 m.mu., the absorption max. of the aromatic **amino acids**, or by the action of cigaret smoke. Solns. of II in III and I in .gamma.-globulin, when irradiated with O,. . . but if III was previously denatured by heat not more than a 15% loss of fluorescence occurred. Addn. of NH3, **cysteine**, or dehydro-.**alpha.-lipoic acid** largely prevented the reaction. The relation of I, **proteins**, and SH groups to carcinogenesis is discussed in the light of these results.
AN 1964:495000 CAPLUS
DN 61:95000
OREF 61:16565g-h,16566a-b
TI Model investigations of the chemical carcinogenesis and of the photodynamic effect of 3,4-benzopyrene and ultraviolet light in aqueous **protein** solutions with different sulfhydryl-group reactivities
AU Reske, Guenter; Stauff, Joachim
CS Univ. Frankfurt/Main, Germany
SO Z. Naturforsch (**1964**), 19b(8), 716-26
DT Journal

LA Unavailable

L7 ANSWER 47 OF 82 CAPLUS COPYRIGHT 2001 ACS
TI Nutritional studies on .**alpha**.-**lipoic acid**
SO Saishin Igaku (**1964**), 19, 224-36
AB In rats fed a low-**protein** diet, in vivo and in vitro incorporation of lipoic acid (I)-35S into **protein**-bound I in liver mitochondria was higher/mg. N than in rats which were fed a normal **protein** diet. The uptake of I-35S into **protein**-bound I in rat liver mitochondria/mg. N was decreased both in vitro and in vivo by deficiency of lysine, tryptophan, and threonine, but not by methionine and **phenylalanine** deficiency. In children, urinary excretion of I/kg. body wt. decreased in nephritis and nephrosis and increased in emaciation. Both in mature and immature rats, a low-**protein** diet decreased the total urinary I/day, serum and liver I/ml. and g., resp., and N and I in liver-cell fractions.. . .
AN 1964:494661 CAPLUS
DN 61:94661
OREF 61:16515b-c
TI Nutritional studies on .**alpha**.-**lipoic acid**
AU Nakamura, Tsuneo; Kusunoki, Tomoichi; Kato, Hidehiko; Konishi, Kiyosaburo; Mibu, Atsuo
CS Med. Coll., Kyoto, Japan
SO Saishin Igaku (**1964**), 19, 224-36
DT Journal
LA Unavailable

L7 ANSWER 48 OF 82 CAPLUS COPYRIGHT 2001 ACS
TI Nutritional studies on cheese starters. I. Vitamin and **amino acid** requirements of single strain starters
SO J. Dairy Res. (**1962**), 29, 63-77
AB A representative selection of single strain starters (Streptococcus cremoris, S. lactis, and S. diacetilactis) was investigated for vitamin and **amino acid** requirements. Niacin, pantothenic acid, and biotin were essential for growth. Biotin was essential even in the presence of oleate (as. . . though it stimulated the growth of some. Under increased O tension (shallow-layer culture) it became essential and was replaceable by .**alpha**.-**lipoic acid** or mevalonic acid. **Amino acid** requirements were detd. by the single omission technique in a synthetic medium based on the concns. of free **amino acids** found in aseptically drawn milk. This medium supported adequate growth, and glutamic acid, **valine**, methionine, **leucine**, **isoleucine**, and histidine proved essential for all strains. Aspartic acid, citrulline, and ornithine were not required by any. All strains of S. cremoris required proline and **phenylalanine**, most of them also required or were stimulated by tyrosine, lysine, and **alanine** and a few by threonine and tryptophan. Only 1 strain required glycine. The **amino acid** requirements of S. lactis (including S. diacetilactis) were much simpler than those for S. cremoris, and except for the requirements by some strains for **arginine** and **phenylalanine**, no addnl. **amino acids** were required. The nutritional requirements correlated well with the other known physiol. characteristics of these species.
AN 1963:10259 CAPLUS
DN 58:10259
OREF 58:1737f-h,1738a
TI Nutritional studies on cheese starters. I. Vitamin and **amino acid** requirements of single strain starters
AU Reiter, B.; Oram, J. D.
CS Natl. Inst. Res. Dairying, Shinfield, Reading, UK
SO J. Dairy Res. (**1962**), 29, 63-77

DT Journal
LA Unavailable

L7 ANSWER 49 OF 82 CAPLUS COPYRIGHT 2001 ACS
SO Ann. N.Y. Acad. Sci. (**1960**), 79, 499-507
AB . . . growth factors supplied by serum, ascitic fluid, or egg yolk. The serum may be replaced by a heat-stable, defatted serum **protein** fraction, cholesterol (I) and Tween 80, or an unsatd. fatty acid. The amt. of I required was reduced upon the. . . lactate required seemed to vary with the amt. of aerobiosis induced. Very little lactate was metabolized. Glucose, or other metabolizable **carbohydrate** appeared to be indispensible. Isotopic expts. revealed that glucose is the precursor of a galactan comprising 10% of the dry. . . 2',3'-nucleotides. Pentose was nonessential. Riboflavine, thiamine, and niacin were required growth factors. Leucovorin and pyridoxal were probably not required, but **.alpha.-lipoic acid** prevented the lessening of growth when crystd. albumin was substituted. GY strain had a partial dependence upon pantothenate and biotin. **Amino acid** mixts. may replace casein digests.
AN 1961:13984 CAPLUS
DN 55:13984
OREF 55:2805h-i,2806a-c
TI Nutrition and metabolism of Mycoplasma mycoides variety mycoides
AU Rodwell, A. W.
CS Animal Health Research Lab., Parkville, Australia
SO Ann. N.Y. Acad. Sci. (**1960**), 79, 499-507
DT Journal
LA Unavailable

L7 ANSWER 50 OF 82 CAPLUS COPYRIGHT 2001 ACS
SO J. Biol. Chem. (**1952**), 197, 851-62
AB . . . to HO2CCH2CH2CHO, which is not oxidized by the enzyme. The oxidase contains approx. 6 moles of bound pyruvic oxidase factor (. **alpha.-lipoic acid**) and 1 mole of diphosphothiamine per mole of **protein** but no diphospho pyridine nucleotide or coenzyme A. Iodoacetate and arsenite at high concns. inhibit the enzyme only slightly in the reaction with ferricyanide. The inhibition by p-chloromercuribenzoate is not reversed by **cysteine** .
AN 1953:3659 CAPLUS
DN 47:3659
OREF 47:649f-h
TI .alpha.-Ketoglutaric oxidase. II. Purification and properties
AU Sanadi, D. R.; Littlefield, J. W.; Bock, Robert M.
CS Univ. of Wisconsin, Madison
SO J. Biol. Chem. (**1952**), 197, 851-62
DT Journal
LA Unavailable

L7 ANSWER 51 OF 82 USPATFULL
TI Use of thiol redox **proteins** for reducing **protein** intramolecular disulfide bonds, for improving the quality of cereal products, dough and baked goods and for inactivating snake, bee and. . .
PI US 6113951 20000905
WO 9308274 19930429
AB Methods of reducing cystine containing animal and plant **proteins** , and improving dough and baked goods' characteristics is provided which includes the steps of mixing dough ingredients with a thiol redox **protein** to form a dough and baking the dough to form a baked good. The method of the present invention preferably. . . with wheat flour which imparts a stronger dough and higher loaf volumes. Methods for reducing snake, bee and scorpion toxin **proteins** with a thiol redox (SH) agent and thereby inactivating the **protein** or

detoxifying the **protein** in an individual are also provided. Protease inhibitors, including the Kunitz and Bowman-Birk trypsin inhibitors of soybean, were also reduced. . . . but was ineffective in reducing the intermolecular disulfides that connect the large to the small subunit. A novel cystine containing **protein** that inhibits pullulanase was isolated; thioredoxin reduction of this **protein** destroyed or greatly reduced its inhibitory activity.

SUMM The present invention relates to the use of thiol redox **proteins** to reduce seed **protein** such as cereal **proteins**, enzyme inhibitor **proteins**, venom toxin **proteins** and the intramolecular disulfide bonds of certain other **proteins**. More particularly, the invention involves use of thioredoxin and glutaredoxin to reduce gliadins, glutenins, albumins and globulins to improve the characteristics of dough and baked goods and create new doughs and to reduce cystine containing **proteins** such as amylase and trypsin inhibitors so as to improve the quality of feed and cereal products. Additionally, the invention involves the isolation of a novel **protein** that inhibits pullulanase and the reduction of that novel **protein** by thiol redox **proteins**. The invention further involves the reduction by thioredoxin of 2S albumin **proteins** characteristic of oil-storing seeds. Also, in particularly the invention involves the use of reduced thiol redox agents to inactivate snake. . .

SUMM Thioredoxin h is also known to reductively activate cytosolic enzyme of **carbohydrate** metabolism, pyrophosphate fructose-6-P, 1-phosphotransferase or PFP (Kiss, F., et al. (1991), Arch. Biochem. Biophys. 287:337-340).

SUMM . . . to reduce thionins in the laboratory (Johnson, T. C., et al. (1987), Plant Physiol. 85:446-451). Thionins are soluble cereal seed **proteins**, rich in cystine. In the Johnson, et al. investigation, wheat purothionin was experimentally reduced by NADPH via NADP-thioredoxin reductase (NTR). . .

SUMM Cereal seeds such as wheat, rye, barley, corn, millet, sorghum and rice contain four major seed **protein** groups. These four groups are the albumins, globulins, gliadins and the glutenins or corresponding **proteins**. The thionins belong to the albumin group or faction. Presently, wheat and rye are the only two cereals from which gluten or dough has been formed. Gluten is a tenacious elastic and rubbery **protein** complex that gives cohesiveness to dough. Gluten is composed mostly of the gliadin and glutenin **proteins**. It is formed when rye or wheat dough is washed with water. It is the gluten that gives bread dough. . .

SUMM Glutenins and gliadins are cystine containing seed storage **proteins** and are insoluble. Storage **proteins** are **proteins** in the seed which are broken down during germination and used by the germinating seedling to grow and develop. Prolamines are the storage **proteins** in grains other than wheat that correspond to gliadins while the glutelins are the storage **proteins** in grains other than wheat that correspond to glutenins. The wheat storage **proteins** account for up to 80% of the total seed **protein** (Kasarda, D. D., et al. (1976), Adv. Cer. Sci. Tech. 1:158-236; and Osborne, T. B., et al. (1893), Amer. Chem.. . . . and therefore the quality of bread. It has been shown from in vitro experiments that the solubility of seed storage **proteins** is increased on reduction (Shewry, P. R., et al. (1985), Adv. Cer. Sci. Tech. 7:1-83). However, previously, reduction of glutenins. . .

SUMM As used herein the term "dough" describes an elastic, pliable **protein** network mixture that minimally comprises a flour, or meal and a liquid, such as milk or water.

SUMM While thioredoxin has been used to reduce albumins in flour, thiol redox **proteins** have not been used to reduce glutenins and gliadins nor

other water insoluble storage **proteins**, nor to improve the quality of dough and baked goods. Thiol redox **proteins** have also not been used to improve the quality of gluten thereby enhancing its value nor to prepare dough from. . .

SUMM Many cereal seeds also contain **proteins** that have been shown to act as inhibitors of enzymes from foreign sources. It has been suggested that these enzyme. . . Biochem. 49:593-626). Two such type enzyme inhibitors are amylase inhibitors and trypsin inhibitors. Furthermore, there is evidence that a barley **protein** inhibitor (not tested in this study) inhibits an .alpha.-amylase from the same source (Weselake, R. J., et al. (1983), Plant Physiol. 72:809-812). Unfortunately, the inhibitor **protein** often causes undesirable effects in certain food products. The trypsin inhibitors in soybeans, notably the Kunitz trypsin inhibitor (KTI) and Bowman-Birk trypsin inhibitor (BBTI) **proteins**, must first be inactivated before any soybean product can be ingested by humans or domestic animals. It is

known that these two inhibitor **proteins** become ineffective as trypsin inhibitors when reduced chemically by sodium borohydride (Birk, Y. (1985), Int. J. Peptide **Protein** Res. 25:113-131, and Birk, Y. (1976), Meth. Enzymol. 45:695-739). These inhibitors like other **proteins** that inhibit proteases contain intramoelcular disulfides and are usually stable to inactivation by heat and proteolysis (Birk (1976), supra.; Garcia-Olmedo,. . . not fully eliminate inhibitor activity. Further, this process is not only expensive but it also destroys many of the other **proteins** which have important nutritional value. For example, while 30 min at 120.degree. C. leads to complete inactivation of the BBTI. . . (Friedman, et al., 1991). The prolonged or higher temperature treatments

required for full inactivation of inhibitors results in destruction of **amino acids** such as cystine, **arginine**, and lysine (Chae, et al., 1984; Skrede and Krogdahl, 1985).

SUMM . . . .alpha.-amylase. Inactivation of inhibitors such as the barley amylase/subtilisin (asi) inhibitor and its equivalent in other cereals by thiol redox **protein** reduction would enable .alpha.-amylases to become fully active sooner than with present procedures, thereby shortening time for malting or similar. . .

SUMM Thiol redox **proteins** have also not previously been used to inactivate trypsin or amylase inhibitor **proteins**. The reduction of trypsin inhibitors such as the Kunitz and Bowman-Birk inhibitor **proteins** decreases their inhibitory effects (Birk, Y. (1985), Int. J. Peptide **Protein** Res. 25:113-131). A thiol redox **protein** linked reduction of the inhibitors in soybean products designed for consumption by humans and domestic animals would require no heat or lower heat than is presently required for **protein** denaturization, thereby cutting the costs of denaturation and improving the quality of the soy **protein**. Also a physiological reductant, a so-called clean additive (i.e., an additive free from ingredients viewed as "harmful chemicals") is highly.

. . industry is searching for alternatives to chemical additives. Further the ability to selectively reduce the major wheat and seed storage **proteins** which are important for flour quality (e.g., the gliadins and the glutenins) in a controlled manner by a physiological reductant such as a thiol redox **protein** would be useful in the baking industry for improving the characteristics of the doughs from wheat and rye and for. . .

SUMM The family of 2S albumin **proteins** characteristic of oil-storing seeds such as castor bean and Brazil nut (Kreis, et al. 1989; Youle and Huang, 1981) which are housed within **protein** bodines in the seed endosperm or cotyledons (Ashton, et al. 1976; Weber,

et al. 1980), typically consist of dissimilar subunits. . . with those of the soybean Bowman-Birk inhibitor (Kreis, et al. 1989) but nothing is known of the ability of 2S **proteins** to undergo

reduction under physiological conditions.
SUMM These 2S albumin **proteins** are rich in methionine. Recently transgenic soybeans which produce Brazil nut 2S **protein** have been generated. Reduction of the 2S **protein** in such soybeans could enhance the integration of the soy **proteins** into a dough network resulting in a soybread rich in methionine. In addition, these 2S **proteins** are often allergens. Reduction of the 2S **protein** would result in the cessation of its allergic activity.
SUMM . . . break down starch in malting and in certain baking procedures carried out in the absence of added sugars or other **carbohydrates**. Obtaining adequate pullulanase activity is a problem especially in the malting industry. It has been known for some time that. . .
SUMM . . . a major concern in several southern and western areas of the United States. Venoms from snakes are characterized by active **protein** components (generally several) that contain disulfide (S--S) bridges located in intramolecular (intrachain) cystines and in some cases in intermolecular (interchain). . . C. (1967) Biochim. Biophys. Acta. 133:346-355; Howard, B. D., et al. (1977) Biochemistry 16:122-125). The neurotoxins of snake venom are **proteins** that alter the release of neurotransmitter from motor nerve terminals and can be presynaptic or postsynaptic. Common symptoms observed in. . .
SUMM The presynaptic neurotoxins are classified into two groups. The first group, the .beta.-neurotoxins, include three different classes of **proteins**, each having a phospholipase A.sub.2 component that shows a high degree of conservation. The **proteins** responsible for the phospholipase A.sub.2 activity have from 6 to 7 disulfide bridges. Members of the .beta.-neurotoxin group are either. . . group. One of these subunits is homologous to the Kunitz-type proteinase inhibitor from mammalian pancreas. The multichain .beta.-neurotoxins have their **protein** components linked ionically whereas the two subunits of .beta.-bungarotoxin are linked covalently by an intermolecular disulfide. The B chain subunit. . .
SUMM . . . enzymatic activity and has two subgroups. The first subgroup, the dendrotoxins, has a single polypeptide sequence of 57 to 60 **amino acids** that is homologous with Kunitz-type trypsin inhibitors from mammalian pancreas and blocks voltage sensitive potassium channels. The second subgroup, such. . .
SUMM . . . S--S groups, but the peptide is unique and does not resemble either phospholipase A.sub.2 or the Kunitz or Kunitz-type inhibitor **protein**. The short neurotoxins (e.g., erabutoxin a and erabutoxin b) are 60 to 62 **amino acid** residues long with 4 intramolecular disulfide bonds. The long neurotoxins (e.g., .alpha.-bungarotoxin and .alpha.-cobratoxin) contain from 65 to 74 residues. . . pharmacological effects, e.g., hemolysis, cytolysis and muscle depolarization. They are less toxic than the neurotoxins. The cytotoxins usually contain 60 **amino acids** and have 4 intramolecular disulfide bonds. The snake venom neurotoxins all have multiple intramolecular disulfide bonds.
SUMM . . . thioredoxin reduced intrachain disulfides in the work done with botulinum A. The tetanus and botulinum A toxins are significantly different **proteins** from the snake neurotoxins in that the latter (1) have a low molecular weight; (2) are rich in intramolecular disulfide. . . other animal proteases; (4) are active without enzymatic modification, e.g., proteolytic cleavage; (5) in many cases show homology to animal **proteins**, such as phospholipase A.sub.2 and Kunitz-type proteases; (6) in most cases lack intermolecular disulfide bonds, and (7) are stable to. . .
SUMM . . . Acta. 133:346-355). These conditions, however, are far from physiological. As defined herein the term "inactivation" with respect to

a toxin **protein** means that the toxin is no longer biologically active in vitro, in that the toxin is unable to link to. . .

SUMM . . . phospholipase A.sub.2, representing respectively 50% and 12% of the total weight of the venom, and minor components such as small **proteins** and peptides, enzymes, amines, and **amino acids**.

SUMM Melittin is a polypeptide consisting of 26 **amino acids** with a molecular weight of 2840. It does not contain a disulfide bridge.

Owing to its high affinity for the lipid-water interphase, the **protein** permeates the phospholipid bilayer of the cell membranes, disturbing its organized structure. Melittin is not by itself a toxin but. . .

SUMM Bee venom phospholipase A.sub.2 is a single polypeptide chain of 128 **amino acids**, is cross-linked by four disulfide bridges, and contains **carbohydrate**. The main toxic effect of the bee venom is due to the strong hydrolytic activity of phospholipase A.sub.2 achieved in. . .

SUMM The other toxic **proteins** in bee venom have a low molecular weight and contain at least two disulfide bridges that seem to play an important structural role. Included are a protease inhibitor (63-65 **amino acids**), MCD or 401-peptide (22 **amino acids**) and apamin (18 **amino acids**).

SUMM . . . polypeptides with three to four disulflde bridges and can be classified in two groups: peptides with from 61 to 70 **amino acids**, that block sodium channel, and peptides with from 36 to 39 **amino acids**, that block potassium channel. The reduction of disulfide bridges on the neurotoxins by nonphysiological reductants such as DTT or .beta.-mercaptoethanol. . .

SUMM It is an object herein to provide a method for reducing a non thionin cystine containing **protein**.

SUMM It is a second object herein to provide methods utilizing a thiol redox **protein** alone or in combination with a reductant or reduction system to reduce glutenins or gliadins present in flour or seeds.

SUMM It is also an object herein to provide methods using a thiol redox **protein** alone or in combination with a reductant or reduction system to improve dough strength and baked goods characteristics such as. . .

SUMM It is a further object herein to provide formulations containing a thiol redox **protein** useful in practicing such methods.

SUMM It is further an object herein to provide a method of reducing an enzyme inhibitor **protein** having disulfide bonds.

SUMM . . . still another object herein is to provide a method of reducing the intramolecular disulfide bonds of a non-thionin, non chloroplast **protein** containing more than one intramolecular cystine comprising adding a thiol redox **protein** to a liquid or substance containing the cystines containing **protein**, reducing the thiol redox **protein** and reducing the cystines containing **protein** by means of the thiol redox **protein**.

SUMM Another object herein is to provide an isolated pullulanase inhibitor **protein** having disulfide bonds and a molecular weight of between 8 to 15 kDa.

SUMM Still another object herein is to provide a method of reducing an animal venom toxic **protein** having one or more intramolecular cystines comprising contacting the cystine containing **protein** with an amount of a thiol redox (SH) agent effective for reducing the **protein**, and maintaining the contact for a time sufficient to reduce one or more disulfide bridges of the one or more intramolecular cystines thereby reducing the neurotoxin **protein**. The thiol redox (SH) agent may be a reduced thioredoxin, reduced lipoic acid in the presence of a thioredoxin, DTT or DTT in the presence of a

thioredoxin and the snake neurotoxin **protein** may be a presynaptic or postsynaptic neurotoxin.

SUMM Still a further object of the invention is to provide a composition comprising a snake neurotoxin **protein** and a thiol redox (SH) agent.

SUMM Still yet another object of the invention is to provide a method of reducing an animal venom toxic **protein** having one or more intramolecular cystines comprising contacting the **protein** with amounts of NADP-thioredoxin reductase, NADPH or an NADPH generator system and a thioredoxin effective for reducing the **protein**, and maintaining the contact for a time sufficient to reduce one or more disulfide bridges of the one or more intramolecular cystines thereby reducing the **protein**.

SUMM . . . the objects of the invention, methods are provided for improving dough characteristics comprising the steps of mixing a thiol redox **protein** with dough ingredients to form a dough and baking said dough.

SUMM Also, in accordance with the objects of the invention, a method is provided for inactivating an enzyme inhibitor **protein** in a grain food product comprising the steps of mixing a thiol redox **protein** with the seed product, reducing the thiol redox **protein** by a reductant or reduction system and reducing the enzyme inhibitor by the reduced thiol redox **protein**, the reduction of the enzyme inhibitor inactivating the enzyme inhibitor.

SUMM The thiol redox **proteins** in use herein can include thioredoxin and glutaredoxin. The thioredoxin includes but is not exclusive of E. coli thioredoxin, thioredoxin. . .

SUMM It should be noted that the invention can also be practiced with **cysteine** containing **proteins**. The **cysteines** can first be oxidized and then reduced via thiol redox **protein** .

DRWD FIG. 1 depicts a graph showing the effect of .alpha.-amylase **protein** inhibitors on activation of NADP-Malate Dehydrogenase in the presence of DTT-reduced Thioredoxin h.

DRWD . . . polyacrylamide electrophoretic gel placed over a long UV wavelength light box showing the Thioredoxin-Linked Reduction of Soluble Sulfur Rich Seed **Proteins**: Durum Wheat .alpha.-Amylase Inhibitor (DSG-1) and Bowman-Birk Soybean Trypsin Inhibitor (BBTI).

DRWD . . . of an SDS polyacrylamide electrophoretic gel placed over a long UV wavelength light box showing the Thioredoxin-Linked Reduction of Seed **Proteins**.

DRWD FIGS. 11A-11B are graphs showing the relative reduction of seed **protein** fractions during germination.

DRWD FIG. 13 is a diagrammatic representation of the proposed role of thioredoxin in forming a **protein** network for bread and pasta.

DRWD FIG. 26 represents photographs of an SDS polyacrylamide electrophoretic gel showing the extent of thioredoxin-linked reduction of myristate-extracted **proteins** from oat flour.

DRWD FIG. 27 represents photographs of an SDS polyacrylamide electrophoretic gel showing the extent of thioredoxin-linked reduction of myristate-extracted **proteins** from triticale flour.

DRWD FIG. 28 represents photographs of an SDS polyacrylamide electrophoretic gel showing the extent of thioredoxin-linked reduction of myristate-extracted **proteins** from rye flour.

DRWD FIG. 29 represents photographs of an SDS polyacrylamide electrophoretic gel showing the extent of thioredoxin-linked reduction of myristate-extracted **proteins** from barley flour.

DRWD . . . 30A-30B represent photographs of an SDS polyacrylamide electrophoretic gel showing the extent of thioredoxin-linked reduction of buffer, ethanol and myristate-extracted **proteins** from teff flour; FIG. 30(a) shows fluorescence and FIG. 30(b) shows the **protein** staining.

DRWD . . . of an SDS polyacrylamide electrophoretic gel showing the effect

of NTS vs. glutathione reductase on the reduction status of myristate-extracted **proteins** from corn, sorghum and rice.

DRWD . . . photograph of an SDS polyacrylamide electrophoretic gel showing the in vivo reduction status and thioredoxin-linked in vitro reduction of myristate-extracted **proteins** from corn, sorghum and rice.

DRWD FIG. 35 represents photographs of an SDS polyacrylamide electrophoretic gel showing the extent of thioredoxin-linked reduction of ethanol-extracted **proteins** from triticale flour.

DRWD FIG. 36 represents photographs of an SDS polyacrylamide electrophoretic gel showing the extent of thioredoxin-linked reduction of ethanol-extracted **proteins** from rye flour.

DRWD FIG. 37 represents photographs of an SDS polyacrylamide electrophoretic gel showing the extent of thioredoxin-linked reduction of ethanol-extracted **proteins** from oat flour.

DRWD FIG. 38 represents photographs of an SDS polyacrylamide electrophoretic gel showing the extent of thioredoxin-linked reduction of ethanol-extracted **proteins** from barley flour.

DRWD . . . 39 represents photographs of an SDS polyacrylamide electrophoretic gels showing the extent of reduction of castor seed matrix and crystalloid **proteins** by various reductants.

DRWD FIG. 40 is a photograph of an SDS polyacrylamide electrophoretic gel showing the reduction specificity of 2S **proteins**.

DRWD FIG. 45 represents photographs of SDS polyacrylamide electrophoretic gels showing the extent of reduction of bee venom **proteins** by various reductants.

DRWD FIG. 46 represents photographs of SDS polyacrylamide electrophoretic gels showing the extent of reduction of scorpion venom **proteins** by various reductants.

DRWD FIG. 47 represents photographs of SDS polyacrylamide electrophoretic gels showing the extent of reduction of snake venom **proteins** by various reductants.

DRWD . . . 48 represents photographs of an SDS polyacrylamide electrophoretic gel showing the extent of reduction of bee, scorpion and snake venom **proteins** with the NTS in the presence and absence of protease inhibitors.

DETD Enzyme Inhibitor **Protein** Experiments

DETD . . . NTR from E. coli ware purchased from American Diagnostics, Inc.

and were also isolated from cells transformed to overexpress each **protein**. The thioredoxin strain containing the recombinant plasmid, PFPI, was kindly provided by Dr. J. -P. Jacquot (de la Motte-Guery, F.. . . Marjorie Russel and Peter Model (Russel, M. et al. (1988) J. Biol. Chem. 263:9015-9019). The Isolation procedure used for these **proteins** was as described in those studies with the following changes: cells were broken in a Ribi cell fractionator at 25,000. . .

DETD CM-1 **protein** was isolated from the albumin-globulin fraction of bread wheat flour as described previously (Kobrehel, K., et al. (1991), Cereal Chem. 68:1-6). A published procedure was also used for the isolation of DSG **proteins** (DSG-1 and DSG-2) from the glutenin fraction of durum wheat (Kobrehel, K. et al. (1989), J. Sci. Food Agric. 48:441-452). The CM-1, DSG-1 and DSG-2 **proteins** were homogeneous in SDS-polyacrylamide gel electrophoresis. Trypsin inhibitors were purchased from Sigma Chemical Co., except for the one from corn kernel which was from Fluca. In all cases, the commercial preparations showed a single **protein** component which migrated as expected in SDS-PAGE (Coomassie Blue stain), but in certain preparations, the band was not sharp.

DETD Other **proteins**

DETD Direct reduction of the **proteins** under study was determined by a modification of the method of Crawford, et al. (Crawford, N. A., et al. (1989),. . . to 70 .mu.l of the buffer solution containing 1 mM NADPH and 10 .mu.g (2 to 17 .mu.M) of target **protein**. When thioredoxin was reduced by dithiothreitol (DTT, 0.5 mM), NADPH and NTR

were omitted. Assays with reduced glutathione were performed. . .

DETD Quantification of labeled **proteins**

DETD To obtain a quantitative indication of the extent of reduction of test **proteins** by the NADP/thioredoxin system, the intensities of their fluorescent bands seen in SDS-polyacrylamide gel electrophoresis were evaluated, using a modification. . . Ultrascan laser densitometer, and the area underneath the peaks was quantitated by comparison to a standard curve determined for each **protein**. For the latter determination, each **protein** (at concentrations ranging from 1 to 5 .mu.g) was reduced by heating for 3 min. at 100.degree. C. in the. . . and excess mBBr derivatized with .beta.-mercaptoethanol. Because the intensity of the fluorescent bands was proportional to the amounts of added **protein**, it was assumed that reduction was complete under the conditions used.

DETD . . . specific thioredoxin in the activation of chloroplast enzymes is one test for the ability of thiol groups of a given **protein** to undergo reversible redox change. Even though not physiological in the case of extraplastidic **proteins**, this test has proved useful in several studies. A case in point is purothionin which, when reduced by thioredoxin h. . . The FBPase, whose physiological activator is thioredoxin f, is unaffected by thioredoxin h. In this Example, the ability of cystine-rich **proteins** to activate FBPase as well as NADP-MDH was tested as set forth above. The .alpha.-amylase inhibitors from durum wheat (DSG-1. . .

DETD CM-1--the bread wheat **protein** that is similar to DSG **proteins** but has a lower molecular weight--also activated NADP-MDH and not FBPase when 20 .mu.g of CM-1 were used as shown. . . that thioredoxin h reduces a variety of .alpha.-amylase inhibitors, which, in turn, activate NADP-MDH in accordance with equations 4-6. These **proteins** were ineffective in enzyme activation when DTT was added in the absence of thioredoxin.

DETD TABLE I

Effectiveness of Thioredoxin-Reduced Trypsin Inhibitors, Thionins, and .alpha.-Amylase Inhibitors in Activating Chloroplast NADP-Malate Dehydrogenase and Fructose Bisphosphatase (DTT.fwdarw.Thioredoxin.fwdarw.Indicated **Protein**.fwdarw.Target Enzyme)

Activation of NADPH-MDH was carried out as in FIG. 1 except that the quantity of DSG or the other **proteins** tested was 20 .mu.g. FBPase activation was tested using the standard DTT assay with 1 .mu.g of E. coli thioredoxin and 20 .mu.g of the indicated **proteins**. The above values are corrected for the limited activation seen with E. coli thioredoxin under these conditions (see FIG. 1).

Protein	M, kDa	No. of S-S Groups	*ACTIVITY, nkat/mg NADP-MDH	FBPase
.alpha.-Amylase Inhibitors				
**DSG-2	17	5	2	0
**DSG-1	14	5	2	0
{CM-1	12	5	12	0
Trypsin Inhibitors				
Cystine-rich. . .				

DETD . . . the reduction of the sulfhydryl reagent, 2',5'-dithiobis(2-nitrobenzoic acid) (DTNB), measured by an increase in absorbance at 412 nm. Here, the **protein** assayed was reduced with NADPH via NTR and a thioredoxin. The DTNB assay proved to be effective for the .alpha.-amylase. . . effective in the DTNB reduction assay, and, as with NADP-MDH activation (Table I), was detectably more active than the DSG **proteins** (See, FIG. 5, conditions were as in FIG. 4 except that the DSG **proteins** were omitted and purothionin .alpha., 20

.mu.g or CM-1, 20 .mu.g was used). The results thus confirmed the enzyme activation. . .

DETD **Protein** Reduction Measurements

DETD . . . and its adaptation for use in plant systems has given a new technique for measuring the sulfhydryl groups of plant **proteins** (Crawford, N. A., et al. (1989), Arch. Biochem. Biophys. 271:223-239). When coupled with SDS-polyacrylamide gel electrophoresis, mBBr can be used to quantitate the change in the sulfhydryl status of redox active **proteins**, even in complex mixtures. This technique was therefore applied to the inhibitor **proteins** to confirm their capacity for reduction by thioredoxin. Here, the test **protein** was reduced with thioredoxin which itself had been previously reduced with either DTT or NADPH and NTR. The mBBr derivative of the reduced **protein** was then prepared, separated from other components by SDS-polyacrylamide gel electrophoresis and its reduction state was examined by fluorescence. In. . . experiments described below, thioredoxin from E. coli was found to be effective in the reduction of each of the targeted **proteins**. Parallel experiments revealed that thioredoxin h and calf thymus thioredoxins reduced, respectively, the **proteins** from seed and animal sources.

DETD . . . of the enzyme activation and dye reduction experiments, DSG-1 was effectively reduced in the presence of thioredoxin. Following incubation the **proteins** were derivatized with mBBr and fluorescence visualized after SDS-polyacrylamide gel electrophoresis (FIG. 6). Reduction was much less with DTT alone. . .

DETD Whereas the major soluble cystine-rich **proteins** of wheat seeds can act as inhibitors of exogenous .alpha.-amylases, the cystine-rich **proteins** of most other seeds lack this activity, and, in certain cases, act as specific inhibitors of trypsin from animal sources. While these **proteins** can be reduced with strong chemical reductants such as sodium borohydride (Birk, Y. (1985), Int. J. Peptide **Protein** Res. 25:113-131, and Birl, Y. (1976), Meth. Enzymol. 45:695-7390), there is little evidence that they can be reduced under physiological. . .

DETD . . . inhibitors from seeds can undergo specific reduction by thioredoxin, the question arose as to whether other types of trypsin inhibitor **proteins** share this property. In the course of this study, several such inhibitors --soybean Kunitz, bovine lung aprotinin, egg white ovoinhibitor and ovomucoid trypsin inhibitors--were tested. While the parameters tested were not as extensive as with the cystine-rich **proteins** described above, it was found that the other trypsin inhibitors also showed a capacity to be reduced specifically by thioredoxin as measured by both the enzyme activation and mBBr/SDS-polyacrylamide gel electrophoresis methods. As was the case for the cystine-rich **proteins** described above, the trypsin inhibitors tested in this phase of the study (soybean Kunitz and animal trypsin inhibitors) activated NADP-MDH. . . that it activated FBPase more effectively than NADP-MDH. It might also be noted that aprotinin resembles certain of the seed **proteins** studied here in that it shows a high content of cystine (ca. 10%) (Kassel, B., et al. (1965), Biochem. Biophys.. . .

DETD The fluorescence evidence for the thioredoxin-linked reduction of one of these **proteins**, the Kunitz inhibitor, is shown in FIG. 7 (highly fluorescent slow moving band). In its reduced form, the Kunitz inhibitor. . .

DETD . . . ability to activate FBPase. The activity differences between these purothionins were unexpected in view of the strong similarity in their **amino acid** sequences (Jones, B. L., et al. (1977), Cereal Chem. 54:511-523) and in their ability to undergo reduction by thioredoxin. A. . .

DETD The above Examples demonstrate that thioredoxin reduces a variety of **proteins**, including .alpha.-amylase, such as the CM and DSG inhibitors, and trypsin inhibitors from seed as well as animal sources.

While. . .

DETD As shown in Table II, the extent of reduction of the seed inhibitor **proteins** by the E. coli NADP/thioredoxin system was time-dependent and reached, depending on the **protein**, 15 to 48% reduction after two hours. The results, based on fluorescence emitted by the major **protein** component, indicate that thioredoxin acts catalytically in the reduction of the .alpha.-amylase and trypsin inhibitors. The ratio of **protein** reduced after two hours to thioredoxin added was greater than one for both the most highly reduced **protein** (soybean Bowman-Birk trypsin inhibitor) and the least reduced **protein** (corn kernel trypsin inhibitor)--i.e., respective ratios of 7 and 2 after a two-hour reduction period. It should be noted that. . .

DETD TABLE II

Extent of Reduction of Seed **Proteins** by the NADP/Thioredoxin System Using the mBBr/SDS-Polyacrylamide Gel Electrophoresis Procedure

The following concentrations of **proteins** were used (nmoles): thioredoxin, 0.08; NTR, 0.01; purothionin-.beta., 1.7; DSG-1, 0.7; corn kernel trypsin inhibitor, 1.0; Bowman-Birk trypsin inhibitor, 1.3; and Kunitz trypsin inhibitor, 0.5. Except for the indicated time difference, other conditions were as in FIG. 6.

Protein	% Reduction After 20 min	120 min
Purothionin-.beta.	15	32
DSG-1	22	38
Corn kernel trypsin inhibitor	3	15
Bowman-Birk trypsin inhibitor	25	48
Kunitz trypsin inhibitor	14	22

DETD Bacteria and animals are known to contain a thiol redox **protein**, glutaredoxin, that can replace thioredoxin in reactions such as ribonucleotide reduction (Holmgren, A. (1985), Annu. Rev. Biochem. 54:237-271). Glutaredoxin is. . .

DETD So far there is no evidence that glutaredoxin interacts with **proteins** from higher plants. This ability was tested, using glutaredoxin from E. coli and the seed **proteins** currently under study. Reduction activity was monitored by the mBBr/SDS polyacrylamide gel electrophoresis procedure coupled with densitometric scanning. It was. . .

DETD The above Examples demonstrate that some of the enzyme inhibitor **proteins** tested can be reduced by glutaredoxin as well as thioredoxin. Those specific for thioredoxin include an .alpha.-amylase inhibitor (DSG-2), and several trypsin inhibitors (Kunitz, Bowman-Birk, aprotinin, and ovomucoid inhibitor). Those **proteins** that were reduced by either thioredoxin or glutaredoxin include the purothionins, two .alpha.-amylase inhibitors (DSG-1, CM-1), a cystine-rich trypsin inhibitor. . .

DETD . . . and FBPase target enzymes shown in Table I are low relative to those seen following activation by the physiological chloroplast **proteins** (thioredoxin m or f), the values shown were found repeatedly and therefore are considered to be real. It seems possible that the enzyme specificity shown by the inhibitor **proteins**, although not relevant physiologically, reflects a particular structure achieved on reduction. It remains to be seen whether such a reduced. . .

DETD . . . physiological consequence of the thioredoxin (or glutaredoxin) linked reduction event is of considerable interest as the function of the targeted **proteins** is unclear. The present results offer a new possibility. The finding that thioredoxin reduces a wide variety of inhibitor **proteins** under physiological conditions suggests that, in the absence of compartmental barriers, reduction can take place within the cell.

DETD . . . analysis of the ability of the treated flour for trypsin activity is made using modifications of the insulin and BAEE (Na-benzoyl-L-**arginine** ethyl ester) assays (Schoellmann, G., et al. (1963), Biochemistry 252:1963; Gonias, S. L., et al. (1983), J. Biol. Chem. 258:14682).. . .

DETD REDUCTION OF CEREAL **PROTEINS**

DETD For isolation of insoluble storage **proteins**, semolina (0.2 g) was extracted sequentially with 1 ml of the following solutions for the indicated times at 25.degree. C.:. . .

DETD In vitro mBBr labelling of **proteins**

DETD . . . unless specified otherwise) were added to 70 .mu.l of this buffer containing 1 mM NADPH and 10 .mu.g of target **protein**. When thioredoxin was reduced by dithiothreitol (DTT), NADPH and NTR were omitted and DTT was added to 0.5 mM. Assays. . .

DETD In vivo mBBr labelling of **proteins**

DETD . . . a microfuge tube. The volume of the suspension was adjusted to 1 ml with the appropriate mBBr or buffer solution. **Protein** fractions of albumin/globulin, gliadin and glutenin were extracted from endosperm of germinated seedlings as described above. The extracted **protein** fractions were stored at -20.degree. C. until use. A buffer control was included for each time point.

DETD . . . Cereal Chem. 62:372-377). A gel solution in 100 ml final volume contained 6.0 g acrylamide, 0.3 g bisacrylamide, 0.024 g **ascorbic acid**, 0.2 mg ferrous sulfate heptahydrate and 0.25 g aluminum lactate. The pH was adjusted to 3.1 with lactic acid. The. . .

DETD . . . in 12% (w/v) trichloroacetic acid and soaked for 4 to 6 hr. with one change of solution to fix the **proteins**; gels were then transferred to a solution of 40% methanol/10% acetic acid for 8 to 10 hr. to remove excess mBBr. The fluorescence of mBBr, both free and **protein** bound, was visualized by placing gels on a light box fitted with an ultraviolet light source (365 nm). Following removal. . .

DETD **Protein** Staining/Destaining/Photography

DETD **Protein** stained gels were photographed with Polaroid type 55 film to produce prints and negatives. Prints were used to determine band. . .

DETD The Polaroid negatives of fluorescent gels and prints of wet **protein** stained gels were scanned with a laser densitometer (Pharmacia-LKB UltroScan XL). Fluorescence was quantified by evaluating peak areas after integration. . .

DETD **Protein** Determination

DETD **Protein** concentrations were determined by the Bradford method (Bradford, M. (1976) Anal. Biochem. 72:248-256), with Bio-Rad reagent and bovine serum albumin. . .

DETD As a result of the pioneering contributions of Osborne and coworkers a century ago, seed **proteins** can be fractionated on the basis of their solubility in aqueous and organic solvents (20). In the case of wheat, preparations of endosperm (flour or semolina) are historically sequentially extracted with four solutions to yield the indicated **protein** fraction: (i) water, albumins; (ii) salt water, globulins; (iii) ethanol/water, gliadins; and (iv) acetic acid/water, glutenins. A wide body of evidence has shown that different **proteins** are enriched in each fraction. For example, the albumin and globulin fractions contain numerous enzymes, and the gliadin and glutenin fractions are in the storage **proteins** required for

germination.

DETD Examples 1, 4 and 5 above describe a number of water soluble seed **proteins** (albumins/globulins, e.g., .alpha.-amylase inhibitors, cystine-rich trypsin inhibitors, other trypsin inhibitors and thionines) that are reduced by the NADP/thioredoxin system, derived either from the seed itself or E. coli. The ability of the system to reduce insoluble storage **proteins** from wheat seeds, viz., representatives of the gliadin and glutenin fractions, is described below. Following incubation with the indicated additions, the gliadin **proteins** were derivatized with mBBr and fluorescence was visualized after SDS-polyacrylamide gel electrophoresis. The lanes in FIG. 8 were as follows:. . . NADPH, reduced glutathione, glutathione reductase (from spinach leaves) and glutaredoxin (from E. coli). 4. NTS: NADPH, NTR, and thioredoxin (both **proteins** from E. coli). 5. MET/T(Ec): .beta.-mercaptoethanol and thioredoxin (E. coli). 6. DTT. 7. DTT/T(Ec): DTT and thioredoxin (E. coli). 8. :DTT/T(W): Same as 7 except with wheat thioredoxin h. 9. NGS,-Gliadin: same as 3 except without the gliadin **protein** fraction. 10. NTS,-Gliadin: same as 4 except without the gliadin **protein** fraction. Based on its reactivity with mBBr, the gliadin fraction was extensively reduced by thioredoxin (FIG. 8). The major members. . . from 25 to 45 kDa. As seen in Examples 1, 4 and 5 with the seed .alpha.-amylase and trypsin inhibitor **proteins**, the gliadins were reduced by either native h or E. coli type thioredoxin (both homogeneous); NADPH (and NTR) or DTT could serve as the reductant for thioredoxin. Much less extensive reduction was observed with glutathione and glutaredoxin--a **protein** able to replace thioredoxin in certain E. coli and mammalian enzyme systems, but not known to occur in higher plants.

DETD The gliadin fraction is made up of four different **protein** types, designated .alpha., .beta., .gamma. and .omega., which can be separated by native polyacrylamide gel electrophoresis under acidic conditions (Bushuk,. . . (S--S) groups and thus has the potential for reduction by thioredoxin. In this study, following incubation with the indicated additions, **proteins** were derivatized with mBBr, and fluorescence was visualized after acidic-polyacrylamide gel electrophoresis. The lanes in FIG. 9 were as follows:. . . leaves) and glutaredoxin (from E. coli). 5. NGS+NTS: combination of 4 and 6. 6. NTS: NADPH, NTR, and thioredoxin (both **proteins** from E. coli). 7. MET/T(Ec): .beta.-mercaptoethanol and thioredoxin (E. coli). 8. DTT/T(Ec): DTT and thioredoxin (E. coli). 9. NTS(-T): same. . .

DETD When the thioredoxin-reduced gliadin fraction was subjected to native gel electrophoresis, the **proteins** found to be most specifically reduced by thioredoxin were recovered in the .alpha. fraction (See, FIG. 9). There was active. . .

DETD The remaining group of seed **proteins** to be tested for a response to thioredoxin--the glutenins--while the least water soluble, are perhaps of greatest interest. The glutenins. . . and semolina (MacRitchie, F., et al. (1990), Adv. Cer. Sci. Tech. 10:79-145). Testing the capability of thioredoxin to reduce the **proteins** of this group was, therefore, a primary goal of the current investigation.

DETD . . . .alpha. .beta. .gamma. Aggregate*

None	22.4	30.4	24.3	29.2
Glutathione	36.4	68.1	60.6	60.1
Glutaredoxin	43.5	83.3	79.7	61.5

Thioredoxin
100.0 100.0 100.0
100.0

*proteins not entering the gel

DETD . . . glutaredoxin. However, in all cases, reduction was greater with thioredoxin and, in some cases, specific to thioredoxin (Table IV, note **proteins** in the 30-40 and 60-110 kDa range). As observed with the other wheat **proteins** tested, both the native h anal E. coli thioredoxins were active and could be reduced with either NADPH and the. . .

DETD . . . the wheat gliadin and glutenin fractions when tested in vitro. The results, however, provide no indication as to whether these **proteins** are reduced in vivo during germination--a question that, to our knowledge, had not been previously addressed (Shutov, A. D., et. . .

DETD To answer this question, we applied the mBBr/SDS-PAGE technique was applied to monitor the reduction status of **proteins** in the germinating seed. We observed that reduction of components in the Osborne fractions increased progressively with time and reached. . . to 3-fold with the albumin/globulins and 5-fold with the glutenins. The results suggest that, while representatives of the major wheat **protein** groups were reduced during germination, the net redox change was greatest with the glutenins.

DETD Although providing new evidence that the seed storage **proteins** undergo reduction during germination, the results of FIG. 11 give no indication as to how reduction is accomplished, i.e., by. . . from in vitro measurements (cf. FIG. 8 and Table IV). For this purpose, the ratio of fluorescence to Coomassie stained **protein** observed in vivo during germination and in vitro with the appropriate enzyme reduction system was calculated. The results shown in. . .

DETD TABLE V

Activities of Enzymes Effecting the Reduction of Thioredoxin h in Semolina (Glucose.fwdarw.Glu-6-P.fwdarw.6-P-Gluconate.fwdarw.NADP.fwdarw.Thioredoxin h)

Protein	Activity (nkat/mg **protein**)
Hexokinase	0.28
Glucose-6-P dehydrogenase	0.45
6-P-Gluconate dehydrogenase	0.39
NTR	0.06
Thioredoxin h	0.35

DETD . . . pathway), thioredoxin h appears to function not only in the activation of enzymes, but also in the mobilization of storage **proteins**.

DETD Dough quality was improved by reducing the flour **proteins** using the NADP/thioredoxin system. Reduced thioredoxin specifically breaks sulfur--sulfur bonds that cross-link different parts of a **protein** and stabilize its folded shape. When these cross-links are cut the **protein** can unfold and link up with other **proteins** in bread, creating an interlocking lattice that forms the elastic network of dough. The dough rises because the network traps. . . and glutenins in flour letting them recombine in a way that strengthened the dough (FIG. 13). Reduced thioredoxin strengthened the **protein** network formed during dough making. For these tests, namely those shown in FIG. 14(c) and FIG. 15(d) (using 10 gm. . .

DETD . . . such as better crumb quality, improved texture and higher loaf volume. Also, based on in vivo analyses with the isolated **proteins**, the native wheat seed NADP/thioredoxin system will also be effective in strengthening the dough.

DETD . . . that yeast for purposes of leavening be added after the reduced thioredoxin has had a chance to reduce the storage **proteins**. The dough is then treated as a regular dough proofed, shaped, etc. and baked.

DETD Reduction of Ethanol-Soluble and Myristate-Soluble Storage **Proteins** from Triticale, Rye, Barley, Oat, Rice, Sorghum, Corn and Teff

DETD . . . and methods used in this Example are according to those set forth above in the section titled "Reduction of Cereal **Proteins**, Materials and Methods."

DETD . . . were added to 70 .mu.L of this buffer containing 1 mM NADPH and 25 to 30 .mu.g of extracted storage **protein**. The ethanol extracted storage **proteins** were obtained by using 50 ml of 70% ethanol for every gm of flour and extracting for 2 hr. In the case of teff, 200 mg of ground seeds were extracted. The myristate extracted **proteins** were obtained by extracting 1 gm of flour with 8 mg sodium myristate in 5 ml of distilled H.sub.2 O. . .

DETD The reactions were carried out in 30 mM Tris-HCl buffer, pH 7.9. When **proteins** were reduced by thioredoxin, the following were added to 70 .mu.L of buffer: 1.2 mM NADPH, to 30 .mu.g of seed **protein** fraction, 0.5 .mu.g E. coli NTR and 1 ug E. coli thioredoxin. For reduction with glutathione, thioredoxin and NTR were. . . of 100 mM 2-mercaptoethanol were added and the samples applied to the gels. In each case, to obtain the extracted **protein**, 1 g ground seeds was extracted with 8 mg of sodium myristate in 5 ml distilled water. With the exception of the initial redox state determination of the **proteins**, samples were extracted for 2 hr at 22.degree. C. and then centrifuged 20 min at 16,000 rpm prior to the. . .

DETD FIGS. 26-30 represent pictures of the gels for the reduction studies of myristate-extracted **proteins** from flour of oat, triticale, rye, barley and teff. Buffer and ethanol-extracted **proteins** are also shown for teff in FIG. 30. In all of the studies represented by FIGS. 26-30, the flour was. . . 20 min. and then with 70% ethanol for 2 hr. Also shown are pictures of the gels for the myristate-extracted **proteins** from corn, sorghum and rice (FIGS. 31 and 32). With corn, sorghum and rice, the ground seeds were extracted only with myristate. Therefore, with corn, sorghum and rice, the myristate extract represents total **protein**, whereas with oat, triticale, rye, barley and teff, the myristate extract represents only the glutenin-equivalent fractions since these flours had. . . gels in FIGS. 26-30, show that the NTS is most effective, as compared to GSH or GSH/GR/NADPH, with myristate-extracted (glutenin-equivalent) **proteins** from oat, triticale, rye, barley and teff. The NTS is also most effective with the total **proteins** from rice (FIGS. 31 and 32). Reduced glutathione is more effective with the total **proteins** from corn and sorghum (FIGS. 31 and 32).

DETD . . . myristate in the presence of mBBr was carried out under a nitrogen atmosphere; in treatment (2), to the myristate extracted **proteins** mBBr was added without prior reduction of the **proteins**; in treatment (3), the myristate extracted **proteins** were reduced by the NADP/thioredoxin system (NTS); in treatment (4) the myristate extracted **proteins** were reduced by NADPH, glutathione and glutathione reductase. As depicted in FIG. 32, treatment (1) is like treatment (2) in. . . and reduced by the NTS and then mBBr was added; and in treatment (4) conditions as in (3) except that **proteins** were reduced by DTT. Treatment (1) in FIG. 31 and treatment (2) in FIG. 32 show the initial redox state of the

proteins in the grains. For all three cereals, the **proteins** in the seed are highly reduced. If extracted in air, the **proteins** become oxidized especially the sorghum and rice. The oxidized **proteins** can be re-reduced, maximally with NTS in all cases. With rice, the reduction is relatively specific for thioredoxin; with corn,. . . glutathione is slightly more effective than thioredoxin. Dithiothretol showed varying effectiveness as a reductant. These experiments demonstrate that the storage **proteins** of these cereals are less specific than in the case of wheat and suggest that thioredoxin should be tested both. . .

DETD . . . hr. The experiment shows that the yeast system is highly active in reducing the two major groups of wheat storage **proteins**.

DETD FIGS. 35-38 represent pictures of gels for the reduction of ethanol-extracted **proteins** from flour of triticale, rye, oat and barley, respectively. The results show that the NTS is most effective with the ethanol-extracted **proteins** from triticale, rye and oat. The ethanol-extracted barley **proteins** are reduced in the control and thioredoxin or glutathione has little effect.

DETD Effect of Thioredoxin-linked Reduction on the Activity and Stability of the Kunitz and Bowman-Birk Soybean Trypsin Inhibitor **Proteins**

DETD . . . from Sigma Chemical Co. (St. Louis, Mo.). E. coli thioredoxin and NTR were isolated from cells transformed to overexpress each **protein**. The thioredoxin strain containing the recombinant plasmid, PFPI, was kindly provided by Dr. J. -P. Jacquot (de La Motte-Guery et. . . was kindly provided by Drs. Marjorie Russel and Peter Model (Russel and Model, 1988). The isolation procedures used for these **proteins** were as described in those studies with the following changes: cells were broken in a Ribi cell fractionator at 25,000. . .

DETD . . . slab gels were scanned with a laser densitometer (Pharmacia-LKB UltraScan XL) and the peak area of the KTI or BBTI **protein** band was obtained by integration with a Pharmacia GelScan XL software program.

DETD . . . Trypsin activity was measured in 50 mM Tris-HCl, pH 7.9, by following the increase in absorbance at 253 nm with N-benzoyl-L-**arginine** ethyl ester as substrate (Mundy et al., 1984) or by the release of azo dye into the trichloroacetic acid (TCA)-soluble. . .

DETD . . . supernatant solution was withdrawn and mixed with 1 ml of 1 N NaOH. The absorbance was read at 440 nm. **Protein** concentration was determined with Bio-Rad reagent using bovine serum albumin as a standard (Bradford, 1976).

DETD . . . specifically by the NADP/thioredoxin system from either E. coli or plants. The reduced forms of glutathione and glutaredoxin (a thiol **protein** capable of replacing thioredoxin in certain animal and bacterial systems, but not known to occur in plants (Holmgren, 1985)) were. . .

DETD . . . 88.9

Reduced by LA/Trx h.sup.3

40.5 87.8

---

*The specific activity of the uninhibited control trypsin was 0.018 .DELTA.A.sub.253 nm /.mu.g/min using Nbenzoyl-L-**arginine** ethyl ester as substrate.

.sup.1 Reduction by E. coli NTS (NADP/thioredoxin system) was conducted a 30.degree. C. for 2 hours.

. . .

DETD Friedman and colleagues observed that heating soybean flour in the presence of sulfur reductants (sodium sulfite, N-acetyl-L-**cysteine**, reduced glutathione, or L-**cysteine**) inactivated trypsin inhibitors, presumably as a result of the reduction or interchange of disulfide groups with other **proteins** in soy flour (Friedman and Gumbmann, 1986; Friedman et al., 1982, 1984).

Inactivation of the trypsin inhibitors by these reductants. . .

DETD Protease inhibitor **proteins** are typically stable to inactivation treatments such as heat. This stability is attributed, at least in part, to the cross-linking. . .

DETD . . . and the proteolytic products were analyzed by SDS-PAGE. The extent of proteolysis was determined by measuring the abundance of intact **protein** on SDS gels by laser densitometer. When tested with a protease preparation from 5-day germinated wheat seeds, the oxidized form. . . reaction that depended on all components of the NADP/thioredoxin system (NTS). BBTI showed the same pattern except that the oxidized **protein** showed greater proteolytic susceptibility relative to KTI. Similar effects were observed with both inhibitors when the plant protease preparation was. . .

DETD This Example shows that reduction by thioredoxin, or dithiothreitol (DTT), leads to inactivation of both **proteins** and to an increase in their heat and protease susceptibility. The results indicate that thioredoxin-linked reduction of the inhibitor **proteins** is relevant both to their industrial processing and to seed germination.

DETD . . . exposed to the protease inhibitors during seed germination, the NADP/thioredoxin system could serve as a mechanism by which the inhibitor **proteins** are modified (inactivated) and eventually degraded (Baumgartner and Chrispeels, 1976; Chrispeels and Baumgartner, 1978; Orf et al., 1977; Wilson, 1988;. . . Yoshikawa et al., 1979). As stated previously, there is evidence that the NADP-thioredoxin system plays a similar role in mobilizing **proteins** during the germination of wheat seeds.

DETD Reduction of Castor Seed 2S Albumin **Protein** by Thioredoxin

DETD The results of the following study of sulfhydryl agents to reduce the 2S **protein** from castor seed (Sharief and Li, 1982; Youle and Huang, 1978) shows that thioredoxin actively reduces intramolecular disulfides of the. . .

DETD . . . from Sigma Chemical Co. (St. Louis, Mo.). E. coli thioredoxin and NTR were isolated from cells transformed to overexpress each **protein**. The thioredoxin strain containing the recombinant plasmid pFPI, was kindly provided by Dr. J. -P. Jacquot (de La Mott-Guery et. . . (Nishizawa et al. 1982), respectively. Thioredoxin h was isolated from wheat seeds by following the procedure devised for the spinach **protein** (Florencio et al. 1988). Glutathione reductase was prepared from spinach leaves (Florencio et al. 1988).

DETD Isolation of **Protein** Bodies

DETD **Protein** bodies were isolated by a nonaqueous method (Yatsu and Jacks, 1968). Shelled dry castor seeds, 15 g, were blended with. . . a JS-20 rotor. After centrifugation, the supernatant fraction was collected and centrifuged 20 min at 41,400.times.g. The pellet, containing the **protein** bodies, was resuspended in 10 ml glycerol and centrifuged as before (41,400.times.g for 20 min) collecting the pellet. This washing. . .

DETD 2S **Protein** Purification Procedure

DETD The 2S **protein** was prepared by a modification of the method of Tully and Beevers (1976). The matrix **protein** fraction was applied to a DEAE-cellulose (DE-52) column equilibrated with 5 mM Tris-HCl buffer, pH 8.5 (Buffer A) and eluted with a 0 to 300 mM NaCl gradient in buffer A. Fractions containing the 2S **protein** were pooled and concentrated by freeze drying. The concentrated fraction was applied to a Pharmacia FPLC Superose-12 (HR 10/30) column equilibrated with buffer A containing 150 mM NaCl. The fraction containing 2S **protein** from the Superose-12 column was applied to an FPLC Mono Q HR 5/5 column equilibrated with buffer A. The column. . . of 0 to 300 mM NaCl in buffer A and finally with buffer A containing 1 M NaCl. The 2S **protein** purified by this method was free of

contaminants in SDS polyacrylamide gels stained with Coomassie blue (Kobrehel et al., 1991).

DETD Reduction of **proteins** was monitored by the monobromobimane (mBBr)/SDS polyacrylamide gel electrophoresis procedure of Crawford et al. (1989). Labeled **proteins** were quantified as described previously in the "Reduction of Cereal **Proteins**, Materials and Methods" section. **Protein** was determined by the method of Bradford (1976).

DETD . . . al., 1981 protocol was used for assaying NADP-malate dehydrogenase and fructose 1,6 bisphosphatase in the presence of thioredoxin and 2S **protein**. Assays were conducted under conditions in which the amount of added thioredoxin was sufficient to reduce the castor 2S **protein** but insufficient to activate the target enzyme appreciably. All assays were at 25.degree. C. Unless otherwise indicated, the thioredoxin and NTR used were from E. coli. The 2S **protein** was monitored during purification by mBBr/SDS-polyacrylamide gel electrophoresis following its reduction by dithiothreitol and E. coli thioredoxin (Crawford et al.,. . .

DETD FIG. 39 represents the reduction of the matrix and crystalloid **proteins** from castor seed as determined by mBBr/SDS-polyacrylamide gel electrophoresis procedure. 1 and 7, Control: no addition; 2 and 8, GSH/GR/NADPH:. . . glutathione, glutathione reductase (from spinach leaves) and glutaredoxin from E. coli; 4 and 10, NTS: NADPH, NTR, and thioredoxin (both **proteins** from E. coli); 5 and 11, NADPH; 6 and 12, NADPH and E. coli NTR. Reactions were carried out in. . . .mu.g NTR and 1 .mu.g thioredoxin were added to 70 .mu.l of this buffer containing 1 mM NADPH and target **protein**: 8 .mu.g matrix **protein** for treatments 1-6 and 10 .mu.g crystalloid **protein** for treatments 7-12. Assays with glutathione were performed similarly, but at a final concentration of 2 mM, 1.4 .mu.g glutathione. . .

DETD FIG. 40 represents the specificity of thioredoxin for reducing the disulfide bonds of castor seed 2S **protein**. (1) Control (no addition); (2) Control+NTS (same conditions as in FIG. 39); (3) Control (heated 3 min at 100.degree. C.); (4) Control+2 mM DTT (heated 3 min at 100.degree. C.). The samples containing 5 .mu.g 2S **protein** and the indicated additions were incubated for 20 min in 30 mM Tris-HCl (pH 7.8). mBBr, 80 nmol, was then. . .

DETD The castor storage **proteins**, which are retained within a **protein** body during seed maturation, can be separated into two fractions on the basis of their solubility. The more soluble **proteins** are housed in the **protein** body outer section ("matrix") and the less soluble in the inner ("crystalloid"). In the current study, the matrix and crystalloid. . . isolated to determine their ability to undergo reduction by cellular thiols, viz., glutathione, glutaredoxin and thioredoxin. Glutaredoxin, a 12 kDa **protein** with a catalytically active thiol group, can replace thioredoxin in certain enzymic reactions of bacteria and animals (Holmgren et al.. . .

DETD FIG. 39 shows that, while a number of storage **proteins** of castor seed were reduced by the thiols tested, only a low molecular weight **protein**, corresponding to the large subunit of the 2S **protein** of the matrix, showed strict specificity for thioredoxin. Certain higher molecular weight **proteins** of the crystalloid fraction underwent reduction, but in those cases there was little difference between glutaredoxin and thioredoxin (FIG. 39). The castor seed 2S large subunit thus appeared to resemble cystine-containing **proteins** previously discussed in undergoing thioredoxin-specific reduction. These experiments were designed to confirm this specificity and to elucidate certain properties of the reduced **protein**. As expected, owing to lack of disulfide groups, the 2S small subunit showed essentially no reaction with mBBr with any. . .

DETD . . . found to depend on all components of the NADP/thioredoxin system (NADPH, NTR and thioredoxin) (Table XIV). As for other thioredoxin-linked **proteins** (including chloroplast enzymes), the thioredoxin active in reduction of the 2S large subunit could be reduced either chemically with dithiothreitol. . . 67% and 90%, respectively, after 20 min at 25.degree. C. Similar, though generally extensive reduction was observed with the disulfide **proteins** discussed above (Johnson et al. 1987). As with the other seed **proteins**, native wheat thioredoxin h and E. coli thioredoxins could be used interchangeably in the reduction of the 2S **protein** by DTT (data not shown).

DETD TABLE XIV

Extent of reduction of the castor castor seed 2S **protein** by different sulfhydryl reductants. Reaction conditions as in FIG. 39. A reduction of 100% corresponds to that obtained when the 2S **protein** was heated for 3 min in the presence of 2% SDS and 2.5% .beta.-mercaptoethanol. NTS: NADPH, NTR, and thioredoxin (both **proteins** from E. coli); GSH/GR/NADPH: reduced glutathione, glutathione reductase (from spinach leaves) and NADPH; NGS: NADPH, reduced glutathione, glutathione reductase (from spinach leaves) and glutaredoxin. . .

DETD The capability of thioredoxin to reduce the castor seed 2S **protein** was also evident in enzyme activation assays. Here, the **protein** targeted by thioredoxin (in this case 2S) is used to activate a thioredoxin-linked enzyme of chloroplasts, NADP-malate dehydrogenase or fructose 1,6-bisphosphatase. As with most of the **proteins** examined so far, the 2S **protein** more effectively activated NADP-malate dehydrogenase and showed little activity with the fructose bisphosphatase (2.6 vs. 0.0 nmoles/min/mg **protein**).

DETD The castor seed 2S **protein** contains inter-as well as intramolecular disulfides. The 2S **protein** thus provides an opportunity to determine the specificity of thioredoxin for these two types of bonds. To this end, the castor seed 2S **protein** was reduced (i) enzymically with the NADP/thioredoxin system at room temperature, and (ii) chemically with DTT at 100.degree. C. Following reaction with mBBr the reduced **proteins** were analyzed by SDS-polyacrylamide gel electrophoresis carried out without additional sulfhydryl agent. The results (FIG. 40) indicate that while thioredoxin.

. .

DETD The present results extend the role of thioredoxin to the reduction of the 2S **protein** of castor seed, an oil producing plant. Thioredoxin specifically reduced the intramolecular disulfides of the large subunit of the 2S **protein** and showed little activity for the intermolecular disulfides joining the large and small subunits. Based on the results with the. . . trypsin inhibitors of soybean, it is clear that reduction of intramolecular disulfides by thioredoxin markedly increases the susceptibility of disulfide **proteins** to proteolysis (Jiao et al. 1992a). It, however, remains to be seen whether reduction of the 2S **protein** takes place prior to its proteolytic degradation (Youle and Huang, 1978) as appears to be the case for the major storage **proteins** of wheat. A related question raised by this work is whether the 2S **protein** of castor, as well as other oil producing plants such as brazil nut (Altenbach et a]., 1987; Ampe et al., 1986), has a function in addition to that of a storage **protein**.

DETD Thioredoxin-Dependent Deinhibition of Pullulanase of Cereals by Inactivation of a Specific Inhibitor **Protein**

DETD . . . at 30,000 g and at 4.degree. C. for 25 min, the supernatant was fractionated by precipitation with solid ammonium sulfate. **Proteins** precipitated between 30% and 60% saturated ammonium

sulfate were dissolved in a minimum volume of 20 mM Tris HCl, pH. . .

DETD . . . centrifuged to remove insoluble materials and the supernatant adjusted to pH 4.6 with 2N formic acid. After pelleting the acid-denatured **protein**, the supernatant was readjusted to pH 7.5 with NH.sub.4 OH and loaded onto a DE52 column (2.5.times.26 cm) equilibrated with. . . 4.6) and Sephacryl-200 HR (30 mM Tris-HCl, pH 7.5, containing 200 mM NaCl and 1 mM EDTA) chromatography. Pullulanase inhibitor **protein** was purified as described below.

DETD . . . centrifugation and the supernatant was chromatographed on a CM32 column (2.5.times.6 cm) equilibrated with 20 mM sodium acetate, pH 4.6. **Proteins** were eluted with a linear 0-0.4 M NaCl in 200 ml of 20 mM sodium acetate, pH 4.6. Fractions (5.0. . .

DETD . . . conducted for the regulation of amylases, little is known about

the regulation of pullulanase in seeds. Yamada (Yamada, J. (1981) **Carbohydrate** Research 90:153-157) reported that incubation of cereal flours with reductants (e.g., DTT) or proteases (e.g., trypsin) led to an activation. . . that is precipitated by ammonium sulfate and inhibits pullulanase. The role of DTT is to reduce and thus inactivate this **protein** inhibitor, leading to activation of pullulanase. Along this line, the 30-60% ammonium sulfate fraction from barley malt was applied to. . . mM Tris-CHl, pH 7.5 (FIG. 41). Following elution with a linear salt gradient, "deinhibited" ("activated") pullulanase was identified as a **protein** peak coming off at about 325 mM NaCl (from fraction numbers 44 to 60). Assay of pullulanase activity in the. . . preincubation mixture consisting of 50 .mu.l of the peak pullulanase activity fraction (fraction number 45) with 50 .mu.l of other **protein** fracitons indicated that a **protein** peak that showed pullulanase inhibitory activity was eluted from the DE52 column by about 100 mM NaCl between fraction numbers. . .

DETD Preliminary experiments showed that pullulanase inhibitor **protein** is resistant to treatment of 70.degree. C. for min and pH 4.0. Based on the profile of Sephadex G-75 gel. . . and SDS-PAGE, pullulanase inhibitor has a molecular weight between 8 to 15 kDa.+-.2 kDa. The study further showed that the **protein** contains thioredoxin-reducible (S--S) bonds.

DETD These studies, as shown in Table XV, found that the ubiquitous dithiol **protein**, thioredoxin, serves as a specific reductant for a previously unknown disulfide-containing **protein** that inhibits pullulanase of barley and wheat endosperm.

DETD TABLE XV

Activity change in Pullulanase Inhibitor **Protein** Following Reduction by NADP/Thioredoxin System

Treatment	Relative Pullulanase Activity
No inhibitor	100
Inhibitor	
Oxidized	30.1
Reduced by DTT	46.1
Reduced by E. coli Trx/DTT	95.1
Reduced by E.. . .	

DETD Reduction of the inhibitor **protein** eliminated its ability to inhibit pullulanase, thereby rendering the pullulanase enzyme active. These studies as shown in Table XV illustrate. . . several sources such as E. coli or seed endosperm (thioredoxin h). The role of thioredoxin in reductively inactivating the inhibitor **protein** (I) and deinhibiting the pullulanase enzyme (E) is given in Equations 1 and 2. ##STR5##

DETD In summary, the crude endosperm extracts were fractionated by column chromatography procedures. These steps served to separate the **protein** inhibitor from the pululanase enzyme. The inhibitor

protein was then highly purified by several steps. By use of the mBBr/SDS-PAGE procedure, it was determined that disulfide group(s) of the new **protein** are specifically reduced by thioredoxin and that the reduced **protein** loses its ability to inhibit pullulanase. Like certain other disulfide **proteins** of seeds (e.g., the Kunitz and Bowman-Birk trypsin inhibitors of soybean), the pullulanase inhibitor **protein** showed the capability to activate chloroplast NADP-malate dehydrogenase. In these experiments, dithiothreitol was used to reduce thioredoxin, which in turn. . .

DETD . . . amino terminus of the pure reductase enzyme is determined by microsequencing by automated Exman degradation with an Applied Biosystems gas-phase **protein** sequencer. On the basis of this sequence, and relying on codon usage in yeast, a 20-base 24-bold degenerate DNA probe. . .

DETD . . . its technological value: (1) by obtaining stronger glutens (increased elasticity, improved extensibility); (2) by increasing gluten yield by capturing soluble **proteins**, reduced by the NADP-thioredoxin system, in the **protein** network, thereby preventing them from being washed out during the production of gluten. In this procedure (using 10 g flour),. . .

DETD The invention provides a method for chemically reducing toxicity causing **proteins** contained in bee, scorpion and snake venome and thereby altering the biological activity of the venoms well as reducing the. . .

DETD . . . the reduced or sulfhydryl (SH) state. As defined herein the term "thiol redox (SH) agent" means a reduced thiol redox **protein** or synthetically prepared agent such as DTT.

DETD . . . Chemical Co. (St. Louis, Mo.). As the phospholipase A.sub.2 was provided in 3.2 M (NH.sub.4).sub.2 SO.sub.4 solution pH 5.5, the **protein** was dialysed in mM Tris-HCl buffer, pH 7.9, using a centricon 3 KDa cutoff membrane. .alpha.-Bungarotoxin and .alpha.-bungarotoxin.sup.125 I were. . .

DETD DL-.**alpha.-Lipoic acid**, L-.alpha.-phosphatidylcholine from soybean, NADPH and .beta.-mercaptoethanol were purchased from Sigma Chemical Co. (St Louis, Mo.) and monobromobimane (mBBr, trade name. . .

DETD **Proteins** and Enzymes

DETD . . . to a solution containing 40% methanol and 10% acetic acid for 12 hr to remove excess mBBr. The fluorescence of **protein**-bound mBBr was determined by placing gels on a light box fitted with an ultraviolet light source (365 nm). Gels were. . . through a yellow Wratten gelatin filter No. 8 (cutoff=460 nm) (exposure time 40 sec. at f4.5). Gels were stained for **protein** for 1 hr in solution of 0.125% (w/v) Coomassie blue R-250 in 10% acetic acid and 40% methanol. Gels were. . .

DETD . . . were boiled for 3 min, and then subjected to SDS-polyacrylamide gel electrophoresis. Gels were stained with Coomassie blue and the **protein** bands quantified by densitometric scanning as described above. The results of the assay are shown in Table XVI below. These. . .

DETD . . . the biological activity and inactivity of animal toxins, namely bee, scorpion and snake toxins. The invention further provides a novel **protein** that is a pullulanase inhibitor and a method for its inactivation.

CLM What is claimed is:

. . . strength and volume of a dough or a baked good comprising the steps of: (a) mixing a reduced thiol redox **protein** selected from the group consisting of thioredoxin and glutaredoxin with dough ingredients containing glutenins or gliadins to form a dough,. . .

2. The method of claim 1 wherein the thiol redox **protein** is

thioredoxin.

. . . of a semolina dough or volume of a cooked pasta comprising the steps of: (a) mixing a reduced thiol redox **protein** selected from the group consisting of thioredoxin and glutaredoxin with semolina dough ingredients containing glutenins or gliadins to form a. . . 7. The method of claim 6 wherein the thiol redox **protein** is thioredoxin.

. . . producing a dough from rice, corn, soybean, barley, oat, sorghum, cassava or millet flour, comprising (a) mixing a thiol redox **protein** selected from the group consisting of thioredoxin and glutaredoxin with said flour to form a mixture, said flour containing storage **proteins**; (b) reducing said thiol redox **protein** by means of thioredoxin reductase and NADPH or an NADPH generating system if said thiol redox **protein** is thioredoxin or glutathione in conjunction with glutathione reductase and NADPH or an NADPH generating system if said thiol redox **protein** is glutaredoxin in said mixture; (c) reducing said storage **proteins** by said reduced thiol redox **protein**, and (d) oxidizing said reduced storage **proteins**, said oxidized storage **proteins** creating a **protein** network complex in the form of a pliable dough.

. . . producing a dough from rice, corn, soybean, barley, oat, sorghum or millet flour, comprising (a) mixing a reduced thiol redox **protein** selected from the group consisting of thioredoxin and glutaredoxin with said flour and a liquid to form a mixture, said flour containing water insoluble storage **proteins**; (b) reducing said storage **proteins** by said reduced thiol redox **protein** , and (c) oxidizing said reduced storage **proteins**, said oxidized storage **proteins** creating a **protein** network complex in the form of a pliable dough.

. . . wheat or rye flour with a liquid to form a mixture, said flour containing glutenins, gliadins and cystine containing soluble **proteins**; (b) adding a thiol redox **protein** selected from the group consisting of thioredoxin and glutaredoxin; (c) reducing said thiol redox **protein** by means of thioredoxin reductase and NADPH or an NADPH generating system if said thiol redox **protein** is thioredoxin or glutathione in conjunction with glutathione reductase and NADPH or an NADPH generating system if said thiol redox **protein** is glutaredoxin; (d) reducing said gliadins, glutenins and soluble **proteins** by said reduced thiol redox **protein**, said reduced glutenins, gliadins and soluble **proteins** forming gluten, and (e) separating said gluten from said mixture.

. . . A method for producing a gluten having increased viscoelasticity comprising (a) mixing a wheat flour with a reduced thiol redox **protein** selected from the group consisting of thioredoxin and glutaredoxin and a liquid, said flour containing glutenins or gliadins; (b) reducing said gliadins and glutenins by said reduced thiol redox **protein**, said reduced glutenins and gliadins forming a gluten with increased viscoelasticity, and (c) separating said gluten from said mixture.

12. A method for producing viscoelastic **protein** comprising (a) mixing a barley, corn, sorghum, rice or millet flour with a liquid to form a mixture, said flour containing water insoluble storage **proteins** and cystine containing soluble **proteins**; (b) adding a thiol redox **protein** selected from the group consisting of thioredoxin and glutaredoxin to said mixture; (c) reducing

said thiol redox **protein** by means of thioredoxin reductase and NADPH or an NADPH generating system if said thiol redox **protein** is thioredoxin or glutathione in conjunction with glutathione reductase and NADPH or an NADPH generating system if said thiol redox **protein** is glutaredoxin; (d) reducing said water insoluble storage **proteins** and soluble **proteins** by said reduced thiol redox **protein,** said reduced **proteins** forming a product that is a sticky, elastic network, and (e) separating said viscoelastic **protein** product from said mixture.

. . . 20. A method of increasing the volume of a cooked pasta comprising the steps of: (a) mixing a thiol redox **protein** selected from the group consisting of reduced thioredoxin and reduced glutaredoxin with pasta dough ingredients to form a dough; (b). . .

. . . 22. A method of increasing the volume of a baked good comprising the steps of: (a) mixing a thiol redox **protein** selected from the group consisting of reduced thioredoxin and reduced glutaredoxin with dough ingredients to form a dough; (b) shaping. . .

29. A composition comprising an intramolecular cystines containing glutenin or gliadin **protein** and added yeast or E. coli thioredoxin, NADP-thioredoxin reductase and NADPH or an NADPH generating system.

30. A method of reducing the intramolecular disulfide bonds of a glutenin or gliadin **protein** containing more than one intramolecular cystine comprising: (a) adding a thiol redox **protein** selected from the group consisting of thioredoxin and glutaredoxin to a liquid or substance containing said cystines containing glutenin or gliadin **protein;** (b) reducing said thiol redox **protein** by means of thioredoxin reductase and NADPH or an NADPH generating system if said thiol redox **protein** is thioredoxin or glutathione in conjunction with glutathione reductase and NADPH or an NADPH generating system if said thiol redox **protein** is glutaredoxin, and (c) reducing said cystines containing glutenin or gliadin **protein** by said reduced thiol redox **protein.**

31. The method of claim 30 wherein the thiol redox **protein** is thioredoxin.

33. The method of claim 31 wherein the thiol redox **protein** is reduced by an NADPH generating system.

34. A method of reducing an a, P or y gliadin comprising (a) adding a thiol redox **protein** selected from the group consisting of thioredoxin and glutaredoxin to a liquid or substance containing said gliadin; (b) reducing said thiol redox **protein** by means of thioredoxin reductase and NADPH or an NADPH generating system if said thiol redox **protein** is thioredoxin or glutathione in conjunction with glutathione reductase and NADPH or an NADPH generating system if said thiol redox **protein** is glutaredoxin, and (c) reducing said gliadin by said reduced thiol redox **protein.**

35. The method of claim 34 wherein the thiol redox **protein** is thioredoxin.

37. The method of claim 34 wherein the thiol redox **protein** is glutaredoxin.

38. A method of reducing a glutenin comprising (a) adding a thiol redox **protein** selected from the group consisting of thioredoxin and glutaredoxin to a liquid or substance containing said glutenin; (b) reducing said thiol redox **protein** by means of thioredoxin reductase and NADPH or an NADPH generating system if said thiol redox **protein** is thioredoxin or glutathione in conjunction with

glutathione reductase and NADPH or an NADPH generating system if said thiol redox **protein** is glutaredoxin, and (c) reducing said glutenin by said reduced thiol redox **protein**.

39. The method of claim 38 wherein the thiol redox **protein** is thioredoxin.

42. The method of claim 38 wherein the thiol redox **protein** is glutaredoxin.

AN 2000:117332 USPATFULL|
TI Use of thiol redox **proteins** for reducing **protein** intramolecular disulfide bonds, for improving the quality of cereal products, dough and baked goods and for inactivating snake, bee and scorpion toxins|
IN Buchanan, Bob B., Berkeley, CA, United States
Kobrehel, Karoly, Montpellier, France
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PA The Regents of the University of California, CA, United States (U.S. corporation)
PI US 6113951 20000905
WO 9308274 19930429
AI US 1994-211673 19941121 (8)
WO 1992-US8595 19921008
19941121 PCT 371 date
19941121 PCT 102(e) date
RLI Continuation-in-part of Ser. No. US 1992-935002, filed on 25 Aug 1992, now abandoned which is a continuation-in-part of Ser. No. US 1991-776109, filed on 12 Oct 1991, now abandoned
DT Utility|
EXNAM Primary Examiner: Sisson, Bradley; Assistant Examiner: Bugaisky, Gabriele E.|
LREP Smith, Karen S.Flehr Hohbach Test Albritton & Herbert LLP|
CLMN Number of Claims: 43|
ECL Exemplary Claim: 1|
DRWN 63 Drawing Figure(s); 53 Drawing Page(s)|
LN.CNT 3550|
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 52 OF 82 USPATFULL
PI US 6087123 20000711
WO 9704007 19970206 <--
SUMM in the **protein** portion they contain among other things the following **protein** sequence:
SUMM . . . are intended to be so understood that RNP also come under this category, in which in the RNA portion and/or **protein** portion exchanges of nucleotides and/or **amino acids** have taken place compared to the sequences shown above, or that only portions of the above sequences are present.
SUMM The invention also relates to DNA, coding for the abovenamed **amino acids**, the DNA comprising:
SUMM typical properties of a ribonucleinic acid (RNA) in the complex of the RNP with polypeptide (**protein**) and copper, zinc and calcium ions,
SUMM typical **protein** properties and **protein** reactions of the polypeptide portion (foline and biuret reaction) in the complex of the RNP with RNA and copper, zinc. . .
SUMM among other things they contain the **amino acids** in the polypeptide portion of the RNP: **alanine** (A), asparagenic acid (D), glutamic acid (E), glycine (G), **isoleucine** (I), lysin (K), **leucine** (L), proline (P), **arginine** (R),

serine (S), theonine (T), **valine** (V), tyrosine (Y);

SUMM no **protein** quaternary structure in the **protein** portion of the RNP in the form of physically bonded peptide sub-units; the native **protein** consists only of a peptide unit (lowest common denominator of the RNP unit);

SUMM . . . favourable for maintaining the life functions of the cells. If however the serum-containing culture solution is to be prepared on **proteins** (mediators), which are generated by the culture, obtaining the product **proteins**, which are normally only present in small concentrations, this presents great difficulties due to the plurality of foreign **proteins** originating from the serum. Moreover, it cannot be ascertained with certainty whether a specific mediator is of humoral or cellular. . .

SUMM . . . fully-synthetic cell culture medium preferably used according to the invention contains the conventional groups of materials, such as salts, sugars, **amino acids**, nucleosides and nucleoside bases, vitamins, vitaminoids, coenzymes and/or steroids in an aqueous solution. It is characterised in that in addition. . .

SUMM . . . or leucocyte culture is preferably used without the addition of serum. Instead of this it receives at least one defined **protein**, which in a particularly preferred embodiment is high-purity, molecularly uniform serum albumin.

SUMM . . . contain further compounds, favourable to the culture of leucocytes, from the classes of materials of the polyhydroxy compounds and sugar, **amino acids**, nucleosides, anionic compounds and/or vitamins, whose use is not conventional in known culture media. The ingredients of the medium used. . .

SUMM . . . 2 KH.sub.2 PO.sub.4 0.2 m

3 NaCl 120.0 m
4 Na.sub.2 HPO.sub.4 0.8 m
5 Na.sub.2 SO.sub.4 0.2 m
6 L-**Ascorbic acid** 0.2 m
7 Cholin chloride 50.0 .mu.
8 2-Desoxy-D-ribose 5.0 .mu.
9 D-Galactose 0.5 m
10 D-Glucose 5.0 m
11. . . m
23 NaHCO.sub.3 10.0 m
24 Human serum albumin 7.7 .mu.
25 Penicillin 1.0 .mu.
26 Streptomycin 1.0 .mu.
27 L-**Glutamine** 1.0 m
28 L-**Alanine** 0.2 m
29 L-Asparagine 0.1 m
30 L-aspartic acid 0.1 m
31 L-glutamic acid 0.1 m
32 glycine 0.2 m
33 L-proline 0.1 m
34 L-serine 0.1 m
35 L-**arginine** 0.4 m
36 4-aminobenzoic acid 2.0 .mu.
37 L-**cysteine** 0.2 m
38 L-hstidine 0.1 m
39 L-hydroxyproline 10.0 .mu.
40 L-**isoleucine** 0.2 m
41 L-**leucine** 0.2 m
42 L-lysine-HCl 0.2 m
43 L-methionine 0.1 m
44 L-ornithine 50.0 .mu.
45 L-**phenylalanine** 0.1 m
46 sarcosine 50.0 .mu.
47 taurine 0.1 m
48 L-threonine 0.2 m

49 L-trypthophane 50.0 .mu.
50 L-tyrosine 0.1 m
51
valine 0.2 m
52 glutathion reduced 3.0 .mu.
53 carnosine 5.0 .mu.
54 mevalolactone 5.0 .mu.
55 adenine 50.0 .mu.
56. . . 1.0 .mu.
68 D-Ca-pantothenate 5.0
69 ergocalciferol 0.5 .mu.
70 D, L-carnitine 50.0 .mu.
71 folic acid 5.0 .mu.
72 D,L-.**alpha**.-**lipoic acid** 2.0 .mu.
73 menadione 0.2 .mu.
74 nicotinic acid amide 20.0 .mu.
75 pyridoxal-HCl 5.0 .mu.
76 pyridoxine-HCl 2.0 .mu.

. . .

SUMM . . . reduction of the volume to be treated. In addition to the small amounts of generated substance, among which are mainly **proteins**, the culture solution contains the mixture of the components of the medium. More advantageously, therefore, in the first step of purification, separation of the resultant **proteins** from the components of the medium and simultaneously from the large volume of aqueous solution, is carried out. This may be effected by a selective salting-out of the **proteins** from the culture solution, which is achieved for example by the addition of a sulphate or phosphate. Thereafter precipitation of the **proteins** is carried out in accordance with the example of salting-out by addition of ammonium sulphate to the culture solution. By means of saturation of the culture solution with ammonium sulphate, the largest proportion of the resultant **proteins**, together with any serum albumin possibly contained, is precipitated out. After separation of the precipitate of substances, for example by. . . the bioactive RNP. The excess is concentrated and the substances obtained are obtained therefrom in the following way. When the **protein**-containing culture solution is mixed with ammonium sulphate up to saturation level, the larger portion of the accompanying **proteins** is precipitated out. In this way a **protein** mixture is obtained which consists of a number of different **proteins** and whose separation into individual components is consequently laborious. In a preferred embodiment of the method according to the invention, the **protein** mixture contained in the culture solution is therefore already separated into a plurality of fractions in the precipitation stage. This separation into a plurality of **protein** fractions is possible, as the individual **proteins** are precipitated out at different ammonium sulphate concentrations. Preferably, the culture solution is mixed, in the method according to the invention, in stages with ammonium sulphate up to specific degrees of saturation, **proteins** being precipitated out in each fraction of the portion, whose solubility product lies beneath the respective degree of saturation. By appropriate selection of the saturation thresholds of the individual fractions, a coarse separation into groups of **proteins** can be achieved during precipitation in the method according to the invention.

SUMM For example, the culture solution is firstly mixed up to a saturation of 35% with ammonium sulphate. The **protein** precipitate obtained in separated. Thereafter the degree of saturation of the residual solution is increased to 45%. A new **protein** precipitate forms,

which is separated. Then the remaining solution is set to a saturation degree of 90%. The **protein** precipitate thus obtained is likewise separated. The solution remaining from this precipitation is for example concentrated by dehydration dialysis or. . .

SUMM Salt precipitation of the **proteins** is carried out likewise, like the following purification, preferably at a temperature of about 0 to 10.degree. C., particularly about. . . a strong buffer, e.g. 0.1 mol/l phosphate buffer, is preferably added. In order to maintain the redox potential of the **proteins**, **cysteine** is preferably added to the solutions in a quantity of 0.001 mol/l. Sterile conditions are not necessary for the **protein** purification.

SUMM The **proteins** obtained during salt precipitation can be passed, after dissolution in a medium which does not damage **proteins**, directly to the purification and separation described hereafter. The residue of the last precipitation stage is concentrated, for example by.

. . In this case all compounds with a molecular weight of greater than about 300 to 500 Daltons, i.e. all the **proteins** and peptides of this fraction, are quantitatively obtained as a dialysis residue.

SUMM The **protein** fractions obtained in the stage described above contain the bioactive RNP according to the invention in a mixture with numerous extraneous **proteins** (other secreted **proteins**, possibly serum, albumin and possibly CON). The extraneous **proteins** are present in a largely predominating amount in the mixtures. By means of a series of purification steps, the bioactive RNP must be enriched and freed from the extraneous **proteins** to such an extent that these latter no longer disturb their molecular biological specificity. The bioactive RNP themselves are likewise. .

SUMM In general, purification processes for **proteins** and other natural products consist of a sequence of combined separating methods, which utilise for separation differences in properties in. . . the accompanying extraneous materials. Accordingly, numerous combinations of the most varied separation methods can be produced for purification of a **protein**. For handling properties, technical feasibility, accessibility to automation and economy of a purification method as well as for the quality. . .

SUMM A plurality of purification stages, known individually per se in biochemistry, are available for purification of the individual **protein** fractions. Examples of such purification steps are: preparative and analytical molecular screen filtration, anion and cation exchanger chromatography, or one-pot. . .

SUMM Even when one of the named purification processes ia carried out only once, a considerable amount of accompanying **proteins** can be separated from the bioactive RNP. However, the substances obtained in the fractions, despite their different molecular weight, frequently. . . separated in accordance with their molecular weight, for example in molecular screen filtration, by the existence of non-ideal equilibriums in **protein** polyelectrolytes. It is therefore recommended to carry out at least two of the named separation processes one after the other. Preferably, the **protein** fractions containing the bioactive RNP are subjected to at least three of the named purification processes in succession.

SUMM Molecular screen filtration causes a separation of **proteins** in accordance with their molecular weight. As a predominating proportion of the accompanying extraneous **proteins** have a different molecular weight from the bioactive RNP, their separation may be achieved in this way. A hydrophilic molecular. . .

SUMM . . . are used with the largest possible particle size, in order to obtain rapid throughflow rates of the frequently rather viscous

protein solutions with the lowest possible pressures. In analytical molecular screen filtration the particle size of the gel matrix is selected. . .

SUMM . . . of a suitable solvent is 0.003 mol/l sodium-potassium phosphate solution with a content of 0.3 mol/l NaCl and 0.001 mol/l **cysteine** and a pH value of 7.4 After filtration, the fractions containing RNP are concentrated in the way described hereafter, and. . .

SUMM . . . 10. A special example of such a buffer solution is 0.01 mol/l tris-HCl, containing 0.04 mol/l NaCl and 0.001 mol/l **cysteine** and having a pH value of 8.0.

SUMM . . . an amount of anion exchanger as is sufficient for total adsorption of the RNP and of the positively adsorbing accompanying **proteins**. More conventionally two portions by volume of swollen anion exchanger per volume of concentrated **protein** fraction are sufficient for this. The reaction can be designed either as a chromatography process or as a more easily handled one-pot adsorption process. In the one-pot process the residual liquid with the negatively adsorbed **proteins** are separated from the anion exchanger charged with the positively adsorbed RNP and other substances, for example by filtration (in. . .

SUMM . . . with RNP freed of negatively adsorbed compounds and other substances is now eluted with an aqueous salt solution harmless to **proteins**, which has an ion strength greater than corresponds to 0.04 mol/l NaCl and a pH value between 4.0 and 10.0.. . . 2.0 mol/l NaCl solution, buffered with 0.01 mol/l hyperazine HCl with a pH value of 6.5, and containing 0.001 mol/l **cysteine**.

SUMM Suitable as cation exchangers for purification of the **protein** fraction are for example dextrane (Sephadex) or cellulose matrices cross-linked with epichloryhydrin, to which are coupled functional groups with cation. . . equilibria, the substance fractions can be diluted before treatment with the cation exchanger with a salt solution harmless to the **proteins**, which has a maximum ion strength equivalent to 0.04 mol NaCl/l. It can simultaneously serve to set the pH value.. . .

SUMM The cation exchanger is added to the substance fraction in a quantity sufficient to adsorb the **protein** fraction. Conventionally sufficient for this is approximately two parts by volume of swelled ion exchanger per part by volume of **protein** fraction. Then the residual liquid is separated from the cation exchanger charged with the substances, for example by decanting or. . .

SUMM . . . exchanger freed of negatively adsorbed compounds, and charged with substances, is now eluated with an aqueous salt solution harmless to **proteins** and nucleinic acids. A salt solution of high ion strength with a pH value of about 4 to 10 is. . .

SUMM . . . onto the hydroxylapatite. Apart from the increase in viscosity due to foreign additives, however, only the phosphate concentration of the **protein** solution is critical for the success of chromatography on hydroxylapatite. Elution of the substances is effected through a sodium phosphate. . .

SUMM . . . method according to the invention to separate by means of appropriate process steps a large proportion of the accompanying extraneous **proteins** from the substance fractions containing the bioactive RNP as traces, before chromatography on hydroxylapatite, and in this way decisively to reduce the **protein** volume which must be applied to the hydroxylapatite column.

SUMM In zone precipitation chromatography (cf. J. Porath, Nature, volume 196 (1962), pages 47-48), **protein** impurities of bioactive RNP are separated by salting-out fractionation of the **proteins** by means of a salt concentration gradient.

SUMM The basic principles of **protein** separation by means of zone precipitation chromatography are various structurally defined reversible

solubility properties of **proteins** and of RNP. They belong to the most sensitive molecular separation parameters and are frequently used as a criteria for proof of the molecular homogeneity of a **protein**. In this case temperatures and pH value, dimensions of the column, type of salt, form of gradient and charge of. . .

SUMM . . . be greater than about 10:1; a ratio of 30 to 100:1 is preferred, particularly about 50:1. All salts harmless to **proteins** and nucleinic acids can be considered in this embodiment. Examples of such salts are sodium-potassium phosphate, ammonium sulphate and sodium. . .

SUMM The salt concentration gradient can be of any optional form, as long as the washing-out points of the **proteins** are separated in terms of the process path. Linear concentration gradients are preferred, particularly a rising linear concentration gradient of. . .

SUMM . . . substance solutions containing bioactive RNP obtained, can be purified of undesired salts and concentrated to form subsequent separations of the **proteins**/RNP. This concentration (separation of the majority of the aqueous salt solution from the substances) can be achieved in various ways.. . . the substance solution. In contrast to higher concentrations, ammonium sulphate in this concentration has an intense salting-in effect relative to **proteins**. By these measures, accordingly, the **proteins** are held in solution during molecular screen filtration. Furthermore, ammonium sulphate prevents bacterial growth and inhibits certain enzymes. In this. . .

SUMM . . . first time easily possible. In order to prevent oxidation, the substance solution is preferably also mixed with about 0.001 mol/l **cysteine**.

SUMM . . . buffered physiological salt solution, for example in 0.0015 mol/l sodium-potassium phosphate solution containing 0.15 mol/l (0.9%) NaCl and 0.001 mol/l **cysteine** and having a pH value of 7.4, after conventional filter sterilisation (pore width 0.2 .mu.m) natively and biologically active also. . .

DETD . . . of the culture solution. All working steps are carried out at 0 to 8.degree. C. in the presence of 0.001 mol/**cysteine**, where not otherwise indicated. Centrifuging is effected as described, either in one or two stages (as continuous flow centrifuging).

DETD . . . The functional viability of the cells is measured on the basis of their motility and stimulability with chemokinetic and chemotactic **proteins**. Mitoses are determined by chromosome count. The morphological viability of the cells at the end of the biotechnical culture is. . .

DETD . . . to remove suspended particles. The clear culture solution obtained (together with 1000 1 with a content of about 1400 g **proteins** and other macromolecules) is directly subjected to the salting-out fractionation with ammonium sulphate.

DETD . . . is mixed with 0.5 mol/l potassium sodium phosphate buffer solution up to a final concentration of 0.1 mol/l. Further, solid L-**cysteine** is added up to a concentration of 0.001 mol/l. The culture solution is then set to an ammonium sulphate saturation. . . the solution is continuously monitored and held at 6.7 by the addition of 2 n ammonia. A portion of the **proteins** is precipitated out of the solution. The **protein** precipitate is separated from the residue containing dissolved substances by centrifuging for 1 hour at 10000.times.g. The **protein** fraction 1 is obtained as a **protein** sludge containing ammonium sulphate, which contains about 100 g **protein**.

DETD . . . solution is continuously monitored and held at 6.7 by the addition of 2 n ammonia. A further proportion of the **proteins** is precipitated out of the solution. The **protein** precipitate is separated from the residue containing dissolved substances by centrifuging for 1 hour at 10000.times.g. The **protein** raw fraction 2 is obtained as a **protein** sludge containing ammonium sulphate, and which contains about 60 g **protein**. The **protein** raw fraction 2 can likewise be separated and, after the

process indicated above, can be processed in order to obtain. . .
DETD . . . solution is continuously monitored and held at 6.7 by the addition of 2 n ammonia. A further proportion of the **proteins** is precipitated out of the solution. The **protein** precipitate is separated from the residue containing dissolved substances by centrifuging for 1 hour at 10000.times.g. The **protein** raw fraction 3 is obtained as a **protein** sludge containing ammonium sulphate, and which contains about 1080 g **proteins**. The majority of the serum albumin is also located in this fraction. The **protein** raw fraction 3 is likewise processed after the process indicated above, in order to obtain its contents. The residue 4 of the raw fraction contains 160 g **proteins** and other macromolecules. Bioactive monocytary RNP is found in this residue.
DETD The residue 4 containing **protein** is diluted with the same volume of buffer solution A (0.15 mol/l NaCl, 0.0015 mol/l potassium-sodium phosphate, 0.001 mol/L-**cysteine**; pH 7.4) to an ammonium sulphate saturation degree of 45% and a phosphate concentration of 0.05 mol/l. This solution is. . .
DETD . . . mixed with a triple volume of its weight of a 0.05 mol/l potassium-sodium phosphate buffer solution, which contains 0.001 mol/l **cysteine** at a pH of 6.80. The suspension obtained is homogenised with a homogeniser (Ultraturax). Then the residue containing the soluble. . .
DETD . . . 25
- - - - (2) INFORMATION FOR SEQ ID NO: 2:
- - (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 91 **amino** - #**acids**
(B) TYPE: **amino acid**
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- - (ii) MOLECULE TYPE: peptide
- - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:. . .
CLM What is claimed is:
. . . of said RNP comprises: AAAGAGAAAGCUGCUCCGAAGNCAG
(SEQ ID NO:1).
the polypeptide portion of said RNP comprises all or part of the **amino acid** sequence: NH.sub.2 -TKLEDHLEGIINIFHQYSVRLG
(SEQ ID NO:3)
- HYDTLIKRELKQLITKELPNTLKN
- TKDQGTIDKIFQNLDANQDEQVSF
- KEFVVLVTDVLITAHDNIHKE-COOH
and wherein the molecular mass of said RNP. . .
. . . of said RNP comprises: AAAGAGAAAGCUGCUCCGAAGNCAG
(SEQ ID NO:1).
the polypeptide portion of said RNP comprises all or part of the **amino acid** sequence: NH.sub.2 -TKLEDHLEGIINIFHQYSVRLG
(SEQ ID NO:3)
- HYDTLIKRELKQLITKELPNTLKN
- TKDQGTIDKIFQNLDANQDEQVSF
- KEFVVLVTDVLITAHDNIHKE-COOH
wherein the molecular mass of said RNP is. . .
. . . mol/l; KH.sub.2 PO.sub.4 0.2 m mol/l; NaCl 120.0 m mol/l; Na.sub.2 HPO.sub.4 0.8 m mol/l; Na.sub.2 SO.sub.4 0.2 m mol/l; L-**Ascorbic acid** 0.2 m mol/l, Cholin Chloride 50.0 .mu.mol/l; 2-Desoxy-D-ribose 5.0 .mu.; D-Galactose 0.5 m mol/l; D-Glucose 5.0 m mol/l; D-Glucurono-.gamma.-lacton 0.1. . . m mol/l; MgCl.sub.2 1.0 m mol/l; NaHCO.sub.3 10.0 mol/l; Human serun albumin 7.7 .mu.mol/l; Penicillin 1.0 .mu.mol/l; Streptomycin 1.0 .mu.mol/l; L-**Glutamine** 1.0 m mol/l; L-**Alanine** 0.2 m mol/l; L-Asparagine 0.1 m mol/l; L-aspartic acid 0.1 m mol/l; L-glutamic acid 0.1 m mol/l; glycine 0.2 m mol/l; L-proline 0.1 m mol/l; L-serine 0.1 m mol/l; L-**arginine** 0.4 m mol/l; 4-aminobenzoic acid 2.0 .mu.mol/l; L-**cysteine** 0.2 m mol/l; L-hstidine 0.1 m mol/l; L-hydroxyproline 10.0 .mu.mol/l; L-**isoleucine** 0.2 m mol/l; L-**leucine** 0.2 m mol/l; L-lysine-HCl 0.2 m mol/l; L-methionine 0.1 m mol/l; L-ornithine 50.0 .mu.mol/l; L-phenylaline 0.1 m mol/l; sacosine

50.0 .mu.mol/l; taurine 0.1 m mol/l; L-threonine 0.2 m mol/l; L-tryptophane 50.0 .mu.mol/l; L-tyrosine 0.1 m mol/l; -**valine** 0.2 m mol/l; glutathion reduced 3.0 .mu.mol/l; carnosine 5.0 .mu.mol/l; mevalolactone 50 .mu.mol/l; adenine 50.0 .mu.mol/l; adenosine 50.0 .mu.mol/l; cytidine. . . .mu.mol/l; xanthine 5.0 .mu.mol/l; biotine 1.0 .mu.mol/l; D-Ca-pantothenate 5.0 mol/l; ergocalciferol 0.5 .mu.mol/l; D,L-carnitine 50.0 .mu.mol/l; folic acid 5.0 .mu.mol/l; D,L-.

alpha.-lipoic acid 2.0 .mu.mol/l; menadione 0.2 .mu.mol/l; nicotinic acid amide 20.0 .mu.mol/l; pyridoxal-HCl5.0 .mu.mol/l; pyridoxine-HCl 2.0 .mu.mol/l; riboflavin 1.0 .mu.mol/l; rutine 5.0. . . acid 5.0 .mu.mol/l; ethanol 1.0 m mol/l; pH7.10; and concanavaline A 50.0 n mol/l; which contains at least one defined **protein**, said **protein** is preferably serum albumin.

. . . culture solution is mixed after termination of the culture with ammonium sulphate up to a saturation of 90%, the precipitated **proteins** are separated from the residue containing ammonium sulphate, the residue is concentrated, purified by preparative molecular screen filtration, an ion. . .

. . . culture solution is mixed after termination of the culture with ammonium sulphate up to a saturation of 35%, the precipitated **proteins** are separated from the residue containing ammonium sulphate, are re-dissolved and purified by an anion exchanger chromatography stage, a preparative. . . chromatography on hydroxylapatite, a zone precipitation chromatography and a cascade molecular screen filtration, and, after separation of the accompanying extraneous **proteins**, the leucocytary RNP is obtained in a highly purified form in the eluate of the cascade molecular screen filtration.

. . . of said RNP comprises: AAAGAGAAAGCUGCUCCGAAGNCAG (SEQ ID NO:1).

the polypeptide portion of said RNP comprises all or part of the **amino acid** sequence: NH.sub.2 -TKLEDHLEGIINIFHQYSVRLG (SEQ ID NO:3)

- HYDTLIKRELKQLITKELPNTLKN
- TKDQGTIDKIFQNLDANQDEQVSF
- KEFVVLVTDVLITAHDNIHKE-COOH

wherein the molecular mass of said RNP is. . .

. . . of said RNP comprises: AAAGAGAAAGCUGCUCCGAAGNCAG (SEQ ID NO:1).

the polypeptide portion of said RNP comprises all or part of the **amino acid** sequence: NH.sub.2 -TKLEDHLEGIINIFHQYSVRLG (SEQ ID NO:3)

- HYDTLIKRELKQLITKELPNTLKN
- TKDQGTIDKIFQNLDANQDEQVSF
- KEFVVLVTDVLITAHDNIHKE-COOH

wherein the molecular mass of said RNP is. . .

. . . mol/l; KH.sub.2 PO.sub.4 0.2 m mol/l; NaCl 120.0 m mol/l; Na.sub.2 HPO.sub.4 0.8 m mol/l; NA.sub.2 SO.sub.4 0.2 m mol/l; L-**Ascorbic acid** 0.2 m mol/l; Cholin Chloride 50.0 .mu.mol/l; 2-Desoxy-D-ribose 5.0 .mu.; D-Galactose 0.5 m mol/l; D-Glucose 5.0 m mol/l; D-Glucurono-.gamma.-lacton 0.1. . . mol/l; MgCl.sub.2 1.0 m mol/l; NaHCO.sub.3 10.0 m mol/l; Human serun albumin 7.7 .mu.mol/l; Penicillin 1.0 .mu.mol/l; Streptomycin 1.0 .mu.mol/l; L-**Glutamine** 1.0 m mol/l; L-**Alanine** 0.2 m mol/l; L-Asparagine 0.1 m mol/l; L-aspartic acid 0.1 m mol/l; L-glutamic acid 0.1 m mol/l; glycine 0.2 m mol/l; L-proline 0.1 m mol/l; L-serine 0.1 m mol/l; L-**arginine** 0.4 m mol/l; 4-aminobeneoic acid 2.0 .mu.mol/l; L-**cysteine** 0.2 m mol/l; L-hstidine 0.1 m mol/l; L-hydroxyproline 10.0 .mu.mol/l; L-**isoleucine** 0.2 m mol/l; L-**leucine** 0.2 m mol/l; L-lysine-HCl 0.2 m mol/l; L-methionine 0.1 m mol/l; L-omithine 50.0 .mu.mol/l; L-phenylaline 0.1 m mol/l; sarcosine

50.0 .mu.mol/l; taurine 0.1 m mol/l; L-threonine 0.2 m mol/l; L-tryptophane 50.0 .mu.mol/l; L-tyrosine 0.1 m mol/l; -**valine** 0.2 m mol/l; glurathion reduced 3.0 .mu.mol/l; carnosine 5.0 .mu.mol/l; mevalolactone 5.0 .mu.mol/l; adenine 50.0 .mu.mol/l; adenosine 50.0 .mu.mol/l; cytidine. . . .mu.mol/l; xanthine 5.0 .mu.mol/l, biotine 1.0 .mu.mol/l; D-Ca-pantothenate 5.0 mol/l; ergocalciferol 0.5 .mu.mol/l; D,L-carninine 50.0 .mu.mol/l; folic acid 5.0 .mu.mol/l; D,L-.

alpha.-lipoic acid 2.0 .mu.mol/l; menadione 0.2 .mu.mol/l; nicotinic acid amide 20.0 .mu.mol/l; pyridoxal-HCl 5.0 .mu.mol/l; pyridoxine-HCl 2.0 .mu.mol/l; riboflavin 1.0 .mu.mol/l; rutine. . . acid 5.0 .mu.mol/l; ethanol 1.0 m mol/l; pH7.10; and concanavaline A 50.0 n mol/l; which contains at least one defined **protein**, said **protein** is preferably serum albumin.

. . . culture solution is mixed after termination of the culture with ammonium sulphate up to a saturation of 90%, the precipitated **proteins** ale separated from the residue containing ammonium sulphate, the residue is concentrated, purified by preparative molecular screen filtration, an ion. . .

. . . culture solution is mixed after termination of the culture with ammonium sulphate up to a saturation of 35%, the precipitated **proteins** are separated from the residue containing ammonium sulphate, are re-dissolved and purified by an anion exchanger chromatography stage, a preparative. . . chromatography on hydroxylapatite, a zone precipitation chromatography and a cascade molecular screen filtration, and, after separation of the accompanying extraneous **proteins**, the leucocytary RNP is obtained in a highly purified form in the eluate of the cascade molecular screen filtration.

AN 2000:87953 USPATFULL|
TI Metal-containing ribonucleotide polypeptides|
IN Wissler, Josef, Bad Nauheim, Germany, Federal Republic of
Logemann, Enno, Freiburg, Germany, Federal Republic of
Kiesewetter, Stefan, Lautertal-Unterlauter, Germany, Federal Republic of
Heilmeyer, Ludwig, Bochum, Germany, Federal Republic of
PA Fraunhofer-Gesellschaft zur Foerderung der Angewandten Forschung e.V., Germany, Federal Republic of (non-U.S. corporation)
PI US 6087123 20000711
WO 9704007 19970206 <--
AI US 1997-794000 19970919 (8)
WO 1996-DE1337 19960717
19970919 PCT 371 date
19970919 PCT 102(e) date
PRAI DE 1995-19525992 19950717
DE 1995-19530500 19950818
DT Utility|
EXNAM Primary Examiner: Elliott, George C.; Assistant Examiner: Shibuya, Mark L.|
LREP Marshall & Melhorn|
CLMN Number of Claims: 46|
ECL Exemplary Claim: 1|
DRWN No Drawings
LN.CNT 1319|
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 53 OF 82 USPATFULL
PI US 5830994 19981103 <--
WO 9508564 19950530 <--
AB Provided is a compound containing a peptide of at least 4 **amino acids** including the following sequence: His Phe* Arg, wherein Phe* represents **phenylalanine** or a halogenated derivative of **phenylalanine** the said peptide being conjugated with thioctic

acid, dihydrolioic acid, or N-lipoyl-lysine, in the form of the corresponding salts, esters. . .

SUMM More particularly, the present invention relates to a compound containing a peptide sequence comprising at least one sequence of 4 **amino acids** obtained from .alpha.-MSH, the **amino acids** being in natural or nonnatural form, the said sequence being conjugated with thioctic acid or a derivative of this acid,. . .

SUMM In this definition, as in the text that will follow, the **amino acids** may be in D, L or D,L form and the nonnatural forms of the **amino acids** correspond to derivatives, especially substituted derivatives.

SUMM Thioctic acid or **.alpha.-lipoic acid** may be in oxidized form: ##STR1## or in the form of a dihydrolipoic derivative: ##STR2##

SUMM These low molecular weight peptides whose **amino acid** sequences have been modified, are linked in the form of salts, esters or amides to active biochemical groups, playing a. . .

SUMM More specifically, the present invention relates to peptides of 4 to 6 **amino acids** linked in the form of Lipoyl-Lysyl-Peptides with anti-allergic, anti-inflammatory and melanogenesis-activating activity.

SUMM in which Phe represents **phenylalanine** or a halogenated derivative of **phenylalanine**, it being possible for the **amino acids** to be in D, L or D,L form, and in particular they may be compounds having the formula:

SUMM Phe is homoPhe or p-fluoroPhe, the **amino acids** being in D, L or D,L form.

SUMM The **amino acid** sequences mentioned above may be natural **amino acid** sequences or nonnatural **amino acid** sequences. Likewise, in some cases it is possible that some of these **amino acids** contain functional groups; for example they are glycosylated and/or sulfated.

SUMM The Peptide III, whose sequence has in the 3-position the **amino acid** paraFluoroPhenyl, is particularly oriented towards an anti-allergic and anti-inflammatory activity, by immunosuppression of Monokines (IL1, IL6, TNF-.alpha.).

SUMM The Peptides II and IV whose sequences possess in the 3- and 2-positions of the **amino acid** D.homoPhenyl are particularly orientated towards a stimulation of the processes of melanogenesis and of Tyrosinase activation.

DETD The **amino acid** derivatives used are:

DETD Each **amino acid** is used in excess (x2) as well as BOP (x2) and each coupling is repeated twice.

DETD Analysis of the **amino acids**:

DETD Analysis of the **amino acids**

CLM What is claimed is:

1. A compound comprising a peptide of at least 4 **amino acids** including the following sequence: His Phe* Arg, wherein Phe* represents **phenylalanine**, homophenylalanine, halogenated **phenylalanine** or halogenated homophenylalanine and the **amino acids** are in the D, L or DL form, the N-terminal of said peptide being conjugated with thioctic acid, dihydrolioic acid,.

. .

2. The compound according to claim 1, characterized in that one or more of the **amino acids** are glycosylated or sulfated.

. . . OH, Trp - Gly - NH2, Trp - NH2, and Trp - OH and Phe* is homoPhe or p-fluoroPhe, the **amino acids** being in D, L or DL form.

5. The compound according to claim 3, characterized in that one or more of the **amino acids** are glycosylated or sulfated.

6. The compound according to claim 4, characterized in that one or more of the **amino acids** are glycosylated or sulfated.

15. The compound of claim 1, wherein Phe* is homophenylalanine, halogenated **phenylalanine** or halogenated homophenylalanine.

17. An anti-allergic or anti-inflammatory compound comprising a peptide of four to six **amino acids** including the following sequence: His Phe* Arg, wherein the amino terminus is acylated with thioctic acid dihydrolipoic acid, or N-lipoyl-lysine, Phe* represents **phenylalanine**, homophenylalanine, halogenated **phenylalanine** or halogenated homophenylalanine, and the **amino acids** are in the D, L, or DL form, and pharmaceutically acceptable salts esters or amides of said peptide.

18. The compound of claim 17, wherein Phe* is homophenylalanine, halogenated **phenylalanine** or halogenated homophenylalanine.

. . . animal an allergy treating or inflammatory reaction treating effective amount of: a compound containing a peptide of at least 4 **amino acids** including the following sequence: His Phe* Arg, wherein Phe* represents **phenylalanine**, homophenylalanine, halogenated **phenylalanine** or halogenated homophenylalanine, the N-terminus of said peptide being conjugated with thioctic acid, dihydrolioic acid, or N-lipoyl-lysine, in the form. . .
. . . 19, wherein an allergy treating or inflammatory reaction treating effective amount of a said compound wherein Phe* is homophenylalanine, halogenated **phenylalanine** or halogenated homophenylalanine.

AN 1998:135138 USPATFULL|
TI Peptide derivatives of alpha-MSH and their application|
IN D'Hinterland, Lucien Dussourd, Toulouse, France
Pinel, Anne-Marie, Toulouse, France
PA Institut Europeen De Biologie Cellulaire, Ramonville-Saint-Agne, France (non-U.S. corporation)
PI US 5830994 19981103 <--
WO 9508564 19950530 <--
AI US 1995-446817 19950522 (8)
WO 1994-FR1108 19940922
19950522 PCT 371 date
19950522 PCT 102(e) date
PRAI FR 1993-11281 19930922
DT Utility|
EXNAM Primary Examiner: Marschel, Ardin H.; Assistant Examiner: Riley, Jezia|
LREP Dechert Price & Rhoads|
CLMN Number of Claims: 22|
ECL Exemplary Claim: 1|
DRWN No Drawings
LN.CNT 1026|
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 54 OF 82 USPATFULL
PI US 5801203 19980901 <--
SUMM This invention relates to the treatment of nervous system disorders, particularly disorders mediated by the N-methyl-D-aspartate (NMDA) subtype of excitatory **amino acid** receptor complex.
SUMM . . . conditions, the patient could be treated prophylactically according to the invention. Other diseases mediated (at least in part) by excitatory **amino acid** toxicity and can be treated by NMDA receptor complex modulation according to the present invention. Such diseases include: 1) ALS. . .
SUMM . . . any nucleophile including an amine; and agents which generate an oxidizing cascade similar to that generated by NO.sup.2 such as . **alpha.-lipoic acid** (thioctic acid and its enantiomers); dihydrolipoate; glutathione; ascorbate; and vitamin E.

SUMM . . . used to enhance absorption into the central nervous system (CNS) and efficacy of SOD and/or catalase. An SOD mimic, the **protein**-bound polysaccharide of Coriolus versicolor QUEL, termed "PS--K", may also be effective by parenteral or oral routes of administration, especially with. . .

DRWD . . . to form an RS--NO (NO.sup.+ equivalent). This chemical reaction leads to a decrease in NMDA receptor-operated channel activation by excitatory **amino acids** (such as NMDA or glutamate) and a concomitant decrease in intracellular calcium influx and amelioration of neurotoxicity.

DRWD . . . above). In addition to glutamate itself, neuronal injury may result from stimulation of the NMDA receptor-channel complex by other excitatory **amino acids**, such as aspartate, quinolinate, homocysteic acid, **cysteine** sulfonic acid, **cysteine**, or from stimulation by excitatory peptides, such as N-acetyl aspartyl glutamate.

DRWD . . . receptor complex-mediated injury, e.g., that injury resulting from stimulation of the NMDA receptor by NMDA(as shown below) or other excitatory **amino acids** or stimulation by excitatory peptides, such as N-acetyl aspartyl glutamate.

DETD . . . experiment of Example 3 was repeated using 1-5 mM NEM, N-ethylmalemide, an agent known to alkylate sulfhydryl (thiol) groups of **proteins**. Following alkylation, neither NTG nor DTNB significantly affected the amplitude of NMDA evoked current, indicating that the redox modulatory site. . .

DETD S-nitrosocysteine (SNOC) both liberates NO.cndot. and participates in nitrosation (NO.sup.+ equivalents reacting with **protein** thiol groups). FIG. 5A is a digital representation of fura-2 calcium images as described above, for 10 cortical neurons in. . .

DETD . . . to enhance their absorption into the CNS and efficacy (Liu et al.,(1989) Am. J. Physiol. 256:589-593. An SOD mimic, the **protein**-bound polysaccharide of Coriolus versicolor QUEL, termed "PS--K", may also be effective by parenteral or oral routes of administration, especially with. . .

DETD TABLE 1

Acute Neurologic Disorders with Neuronal Damage Thought to be Mediated at Least in Part by Excitatory **Amino Acids***

i.	domoic acid poisoning from contaminated mussels
ii.	cerebral ischemia, stroke
iii.	hypoxia, anoxia, carbon monoxide poisoning
iv.	hypoglycemia
v.	prolonged epileptic seizures
vi.	mechanical trauma. . .

DETD TABLE 2

Chronic Neurodegenerative Diseases with Neuronal Damage Thought or Proposed to be Mediated at Least in Part by Excitatory **Amino Acids**.*

i.	Neurolathyrisin-BOAA (.beta.-N-oxalylamino-L-**alanine**) in chick peas
ii.	Guam Disease-BMAA (.beta.-N-methyl-amino-L-**alanine**) in flour from cycad seeds
iii.	Hungtington's disease
iv.	ALS (amyotrophic lateral sclerosis)
v.	Parkinsonism
vi.	Alzheimer's disease
vii.	AIDS dementia complex (HIV-associated cognitive/motor complex)
viii..	. .

DETD TABLE 3

Nitric Oxide Synthase Inhibitors:

1. **Arginine** analogs including N-mono-methyl-L-**arginine** (NMA)
2. N-amino-L-**arginine** (NAA)
3. N-nitro-L-**arginine** (NNA)
4. N-nitro-L-**arginine** methyl ester
5 N-iminoethyl-L-ornithine
6. Diphenylene iodonium and analogs
See, Steuhr, FASEB J 5:98-103 (1991)
7. Diphenyliodonium, calmodulin inhibitors such as trifluoparizine, calmidazolium. . .

AN 1998:104773 USPATFULL
TI Nitroglycerine patch
IN Lipton, Stuart A., Newton, MA, United States
PA The Children's Medical Center Corporation, Boston, MA, United States (U.S. corporation)
PI US 5801203 19980901 <--
AI US 1995-482365 19950607 (8)
RLI Continuation of Ser. No. US 1993-25028, filed on 2 Mar 1993, now patented, Pat. No. US 5455279 which is a continuation-in-part of Ser. No. US 1992-949342, filed on 22 Sep 1992, now patented, Pat. No. US 5234956 which is a continuation of Ser. No. US 1991-688965, filed on 19 Apr 1991, now abandoned
DT Utility
EXNAM Primary Examiner: Criares, Theodore J.
LREP Fish & Richardson P.C.
CLMN Number of Claims: 1
ECL Exemplary Claim: 1
DRWN 12 Drawing Figure(s); 10 Drawing Page(s)
LN.CNT 923
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 55 OF 82 USPATFULL
PI US 5792506 19980811 <--
AB Thioredoxin, a small dithiol **protein**, is a specific reductant for major allergenic **proteins** present in widely used foods from animal and plant sources. All targeted allergenic **proteins** contain disulfide (S--S) bonds that are reduced to the sulfhydryl (SH) level by thioredoxin. The **proteins** are allergenically active in the oxidized (S--S) state. When reduced (SH state), they lose their allergenicity. Thioredoxin achieved this reduction. . .
SUMM The present invention relates to the use of thiol redox **proteins** to reduce seed **protein** such as cereal **proteins**, enzyme inhibitor **proteins**, venom toxin **proteins** and the intramolecular disulfide bonds of certain other **proteins**. More particularly, the invention involves use of thioredoxin and glutaredoxin to reduce gliadins, glutenins, albumins and globulins to improve the characteristics of dough and baked goods and create new doughs and to reduce cystine containing **proteins** such as amylase and trypsin inhibitors so as to improve the quality of feed and cereal products. Additionally, the invention involves the isolation of a novel **protein** that inhibits pullulanase and the reduction of that novel **protein** by thiol redox **proteins**. The invention further involves the reduction by thioredoxin of 2S albumin **proteins** characteristic of oil-storing seeds. Also, the invention involves inactivating snake neurotoxins and certain insect and scorpion venom toxins in vitro. . .
SUMM Thioredoxin h is also known to reductively activate cytosolic enzyme of **carbohydrate** metabolism, pyrophosphate fructose-6-P, 1-phosphotransferase or PFP (Kiss, F., et al. (1991), Arch. Biochem. Biophys. 287:337-340).
SUMM . . . to reduce thionins in the laboratory (Johnson, T. C., et al.

(1987), Plant Physiol. 85:446-451). Thionins are soluble cereal seed **proteins**, rich in cystine. In the Johnson, et al. investigation, wheat purothionin was experimentally reduced by NADPH via NADP-thioredoxin reductase (NTR). . . 2 and 3. ##STR2## Cereal seeds such as wheat, rye, barley, corn, millet, sorghum and rice contain four major seed **protein** groups. These four groups are the albumins, globulins, gliadins and the glutenins or corresponding **proteins** . The thionins belong to the albumin group or faction. Presently, wheat and rye are the only two cereals from which gluten or dough has been formed. Gluten is a tenacious elastic and rubbery **protein** complex that gives cohesiveness to dough. Gluten is composed mostly of the gliadin and glutenin **proteins**. It is formed when rye or wheat dough is washed with water. It is the gluten that gives bread dough. . .

SUMM Glutenins and gliadins are cystine containing seed storage **proteins** and are insoluble. Storage **proteins** are **proteins** in the seed which are broken down during germination and used by the germinating seedling to grow and develop. Prolamines are the storage **proteins** in grains other than wheat that correspond to gliadins while the glutelins are the storage **proteins** in grains other than wheat that correspond to glutenins. The wheat storage **proteins** account for up to 80% of the total seed **protein** (Kasarda, D. D., et al. (1976), Adv. Cer. Sci. Tech. 1:158-236; and Osborne, T. B., et al. (1893), Amer. Chem.. . . and therefore the quality of bread. It has been shown from in vitro experiments that the solubility of seed storage **proteins** is increased on reduction (Shewry, P. R., et al. (1985), Adv. Cer. Sci. Tech. 7:1-83). However, previously, reduction of glutenins. . .

SUMM As used herein the term "dough" describes an elastic, pliable **protein** network mixture that minimally comprises a flour, or meal and a liquid, such as milk or water.

SUMM While thioredoxin has been used to reduce albumins in flour, thiol redox **proteins** have not been used to reduce glutenins and gliadins nor other water insoluble storage **proteins**, nor to improve the quality of dough and baked goods. Thiol redox **proteins** have also not been used to improve the quality of gluten thereby enhancing its value nor to prepare dough from. . .

SUMM Many cereal seeds also contain **proteins** that have been shown to act as inhibitors of enzymes from foreign sources. It has been suggested that these enzyme. . . Biochem. 49:593-626). Two such type enzyme inhibitors are amylase inhibitors and trypsin inhibitors. Furthermore, there is evidence that a barley **protein** inhibitor (not tested in this study) inhibits an a-amylase from the same source (Weselake, R. J., et al. (1983), Plant Physiol. 72:809-812). Unfortunately, the inhibitor **protein** often causes undesirable effects in certain food products. The trypsin inhibitors in soybeans, notably the Kunitz trypsin inhibitor (KTI) and Bowman-Birk trypsin inhibitor (BBTI) **proteins**, must first be inactivated before any soybean product can be ingested by humans or domestic animals. It is known that these two inhibitor **proteins** become ineffective as trypsin inhibitors when reduced chemically by sodium borohydride (Birk, Y. (1985), Int. J. Peptide **Protein** Res. 25:113-131, and Birk, Y. (1976), Meth. Enzymol. 45:695-739). These inhibitors like other **proteins** that inhibit proteases contain intramoelcular disulfides and are usually stable to inactivation by heat and proteolysis (Birk (1976), supra.; Garcia-Olmedo,. . . not fully eliminate inhibitor activity. Further, this process is not only expensive but it also destroys many of the other **proteins** which have important nutritional value. For example, while 30 min at 120.degree. C. leads to complete inactivation of the BBTI. . . (Friedman, et al., 1991). The prolonged or higher temperature treatments

required for full inactivation of inhibitors results in destruction of **amino acids** such as cystine, **arginine**, and lysine (Chae, et al., 1984; Skrede and Krogdahl, 1985).

SUMM . . . .alpha.-amylase. Inactivation of inhibitors such as the barley amylase/subtilisin (asi) inhibitor and its equivalent in other cereals by thiol redox **protein** reduction would enable .alpha.-amylases to become fully active sooner than with present procedures, thereby shortening time for malting or similar. . .

SUMM Thiol redox **proteins** have also not previously been used to inactivate trypsin or amylase inhibitor **proteins**. The reduction of trypsin inhibitors such as the Kunitz and Bowman-Birk inhibitor **proteins** decreases their inhibitory effects (Birk, Y. (1985), Int. J. Peptide **Protein** Res. 25:113-131). A thiol redox **protein** linked reduction of the inhibitors in soybean products designed for consumption by humans and domestic animals would require no heat or lower heat than is presently required for **protein** denaturization, thereby cutting the costs of denaturation and improving the quality of the soy **protein**. Also a physiological reductant, a so-called clean additive (i.e., an additive free from ingredients viewed as "harmful chemicals") is highly.

. . industry is searching for alternatives to chemical additives. Further the ability to selectively reduce the major wheat and seed storage **proteins** which are important for flour quality (e.g., the gliadins and the glutenins) in a controlled manner by a physiological reductant such as a thiol redox **protein** would be useful in the baking industry for improving the characteristics of the doughs from wheat and rye and for. . .

SUMM The family of 2S albumin **proteins** characteristic of oil-storing seeds such as castor bean and Brazil nut (Kreis, et al. 1989; Youle and Huang, 1981) which are housed within **protein** bodines in the seed endosperm or cotyledons (Ashton, et al. 1976; Weber, et al. 1980), typically consist of dissimilar subunits. . . with those of the soybean Bowman-Birk inhibitor (Kreis, et al. 1989) but nothing is known of the ability of 2S **proteins** to undergo reduction under physiological conditions.

SUMM These 2S albumin **proteins** are rich in methionine. Recently transgenic soybeans which produce Brazil nut 2S **protein** have been generated. Reduction of the 2S **protein** in such soybeans could enhance the integration of the soy **proteins** into a dough network resulting in a soybread rich in methionine. In addition, these 2S **proteins** are often allergens. Reduction of the 2S **protein** would result in the cessation of its allergic activity.

SUMM . . . break down starch in malting and in certain baking procedures carried out in the absence of added sugars or other **carbohydrates**. Obtaining adequate pullulanase activity is a problem especially in the malting industry. It has been known for some time that. . .

SUMM . . . a major concern in several southern and western areas of the United States. Venoms from snakes are characterized by active **protein** components (generally several) that contain disulfide (S--S) bridges located in intramolecular (intrachain) cystines and in some cases in intermolecular (interchain). . . C. (1967) Biochim. Biophys. Acta. 133:346-355; Howard, B. D., et al. (1977) Biochemistry 16:122-125). The neurotoxins of snake venom are **proteins** that alter the release of neurotransmitter from motor nerve terminals and can be presynaptic or postsynaptic. Common symptoms observed in. . . individual, etc. The presynaptic neurotoxins are classified into two groups. The first group, the .beta.-neurotoxins, include three different classes of **proteins**, each having a phospholipase A.sub.2 component that shows a high degree of conservation. The **proteins** responsible for the phospholipase A.sub.2 activity have from 6 to 7 disulfide bridges. Members of the .beta.-neurotoxin group are either.

. group. One of these subunits is homologous to the Kunitz-type proteinase inhibitor from mammalian pancreas. The multichain .beta.-neurotoxins have their **protein** components linked ionically whereas the two subunits of .beta.-bungarotoxin are linked covalently by an intermolecular disulfide. The B chain subunit. . .

SUMM . . . enzymatic activity and has two subgroups. The first subgroup, the dendrotoxins, has a single polypeptide sequence of 57 to 60 **amino acids** that is homologous with Kunitz-type trypsin inhibitors from mammalian pancreas and blocks voltage sensitive potassium channels. The second subgroup, such. . .

SUMM . . . S--S groups, but the peptide is unique and does not resemble either phospholipase A.sub.2 or the Kunitz or Kunitz-type inhibitor **protein**. The short neurotoxins (e.g., erabutoxin a and erabutoxin b) are 60 to 62 **amino acid** residues long with 4 intramolecular disulfide bonds. The long neurotoxins (e.g., .alpha.-bungarotoxin and .alpha.-cobratoxin) contain from 65 to 74 residues. . . pharmacological effects, e.g., hemolysis, cytolysis and muscle depolarization. They are less toxic than the neurotoxins. The cytotoxins usually contain 60 **amino acids** and have 4 intramolecular disulfide bonds. The snake venom neurotoxins all have multiple intramolecular disulfide bonds.

SUMM . . . thioredoxin reduced intrachain disulfides in the work done with botulinum A. The tetanus and botulinum A toxins are significantly different **proteins** from the snake neurotoxins in that the latter (1) have a low molecular weight; (2) are rich in intramolecular disulfide. . . other animal proteases; (4) are active without enzymatic modification, e.g., proteolytic cleavage; (5) in many cases show homology to animal **proteins**, such as phospholipase A.sub.2 and Kunitz-type proteases; (6) in most cases lack intermolecular disulfide bonds, and (7) are stable to. . .

SUMM . . . Acta. 133:346-355). These conditions, however, are far from physiological. As defined herein the term "inactivation" with respect to a toxin **protein** means that the toxin is no longer biologically active in vitro, in that the toxin is unable to link to. . .

SUMM . . . phospholipase A.sub.2, representing respectively 50% and 12% of the total weight of the venom, and minor components such as small **proteins** and peptides, enzymes, amines, and **amino acids**.

SUMM Melittin is a polypeptide consisting of 26 **amino acids** with a molecular weight of 2840. It does not contain a disulfide bridge.

Owing to its high affinity for the lipid-water interphase, the **protein** permeates the phospholipid bilayer of the cell membranes, disturbing its organized structure. Melittin is not by itself a toxin but. . .

SUMM Bee venom phospholipase A.sub.2 is a single polypeptide chain of 128 **amino acids**, is cross-linked by four disulfide bridges, and contains **carbohydrate**. The main toxic effect of the bee venom is due to the strong hydrolytic activity of phospholipase A.sub.2 achieved in. . .

SUMM The other toxic **proteins** in bee venom have a low molecular weight and contain at least two disulfide bridges that seem to play an important structural role. Included are a protease inhibitor (63-65 **amino acids**), MCD or 401-peptide (22 **amino acids**) and apamin (18 **amino acids**).

SUMM . . . polypeptides with three to four disulflde bridges and can be classified in two groups: peptides with from 61 to 70 **amino acids**, that block sodium channel, and peptides with from 36 to 39 **amino acids**, that block potassium channel. The reduction of disulfide bridges on the neurotoxins by nonphysiological

reductants such as DTT or .beta.-mercaptoethanol. . .

SUMM Many of the major allergenic **proteins** in the above mentioned foods have intramolecular disulfide (S--S) bonds but so far two treatments have been applied commercially to. . . partial. While lowering allergenicity, heat treatment has not eliminated the problem, even in the best of cases, because the responsible **proteins** are typically heat stable. Moreover, heat lowers product quality by destroying nutritionally important **amino acids** such as lysine, **cysteine** and **arginine**. Enzymatic proteolysis is more successful in reducing allergenicity, but desirable food properties such as flavor are usually lost and treatment. . .

SUMM It is an object herein to provide a method for reducing a non thionin cystine containing **protein**.

SUMM It is a second object herein to provide methods utilizing a thiol redox **protein** alone or in combination with a reductant or reduction system to reduce glutenins or gliadins present in flour or seeds.

SUMM It is also an object herein to provide methods using a thiol redox **protein** alone or in combination with a reductant or reduction system to improve dough strength and baked goods characteristics such as. . .

SUMM It is a further object herein to provide formulations containing a thiol redox **protein** useful in practicing such methods.

SUMM It is further an object herein to provide a method of reducing an enzyme inhibitor **protein** having disulfide bonds.

SUMM . . . still another object herein is to provide a method of reducing the intramolecular disulfide bonds of a non-thionin, non chloroplast **protein** containing more than one intramolecular cystine comprising adding a thiol redox **protein** to a liquid or substance containing the cystines containing **protein**, reducing the thiol redox **protein** and reducing the cystines containing **protein** by means of the thiol redox **protein**.

SUMM Another object herein is to provide an isolated pullulanase inhibitor **protein** having disulfide bonds and a molecular weight of between 8 to 15 kDa.

SUMM Still another object herein is to provide a method of reducing an animal venom toxic **protein** having one or more intramolecular cystines comprising contacting the cystine containing **protein** with an amount of a thiol redox (SH) agent effective for reducing the **protein**, and maintaining the contact for a time sufficient to reduce one or more disulfide bridges of the one or more intramolecular cystines thereby reducing the neurotoxin **protein**. The thiol redox (SH) agent may be a reduced thioredoxin, reduced lipoic acid in the presence of a thioredoxin, DTT or DTT in the presence of a thioredoxin and the snake neurotoxin **protein** may be a presynaptic or postsynaptic neurotoxin.

SUMM Still a further object of the invention is to provide a composition comprising a snake neurotoxin **protein** and a thiol redox (SH) agent.

SUMM Still yet another object of the invention is to provide a method of reducing an animal venom toxic **protein** having one or more intramolecular cystines comprising contacting the **protein** with amounts of NADP-thioredoxin reductase, NADPH or an NADPH generator system and a thioredoxin effective for reducing the **protein**, and maintaining the contact for a time sufficient to reduce one or more disulfide bridges of the one or more intramolecular cystines thereby reducing the **protein**.

SUMM . . . the objects of the invention, methods are provided for improving dough characteristics comprising the steps of mixing a thiol redox **protein** with dough ingredients to form a dough and baking said dough.

SUMM Also, in accordance with the objects of the invention, a method is provided for inactivating an enzyme inhibitor **protein** in a grain food product comprising the steps of mixing a thiol redox

protein with the seed product, reducing the thiol redox **protein** by a reductant or reduction system and reducing the enzyme inhibitor by the reduced thiol redox **protein**, the reduction of the enzyme inhibitor inactivating the enzyme inhibitor.

SUMM The thiol redox **proteins** in use herein can include thioredoxin and glutaredoxin. The thioredoxin includes but is not exclusive of E. coli thioredoxin, thioredoxin. . .

SUMM It should be noted that the invention can also be practiced with **cysteine** containing **proteins**. The **cysteines** can first be oxidized and then reduced via thiol redox **protein** .

SUMM . . . in accordance with the objects of the invention, a method is provided for decreasing the allergenicity of an allergenic food **protein** comprising the steps of contacting the **protein** with an amount of thioredoxin, NTR and NADPH or an amount of DTT in the presence of thioredoxin effective for decreasing the allergenicity of the **protein** and administering the contacted **protein** in step (a) to an animal, thereby decreasing the allergenic symptoms in said animal that would otherwise occur if the animal received the untreated **protein**.

DETD Enzyme Inhibitor **Protein** Experiments Starting Materials

DETD . . . NTR from E. coli ware purchased from American Diagnostics, Inc.

and were also isolated from cells transformed to overexpress each **protein**. The thioredoxin strain containing the recombinant plasmid, pFP1, was kindly provided by Dr. J.-P. Jacquot (de la Motte-Guery, F. et. . . Marjorie Russel and Peter Model (Russel, M. et al. (1988) J. Biol. Chem. 263:9015-9019). The Isolation procedure used for these **proteins** was as described in those studies with the following changes: cells were broken in a Ribi cell fractionator at 25,000. . .

DETD CM-1 **protein** was isolated from the albumin-globulin fraction of bread wheat flour as described previously (Kobrehel, K., et al. (1991), Cereal Chem. 68:1-6). A published procedure was also used for the isolation of DSG **proteins** (DSG-1 and DSG-2) from the glutenin fraction of durum wheat (Kobrehel, K. et al. (1989), J. Sci. Food Agric. 48:441-452). The CM-1, DSG-1 and DSG-2 **proteins** were homogeneous in SDS-polyacrylamide gel electrophoresis. Trypsin inhibitors were purchased from Sigma Chemical Co., except for the one from corn kernel which was from Fluca. In all cases, the commercial preparations showed a single **protein** component which migrated as expected in SDS-PAGE (Coomassie Blue stain), but in certain preparations, the band was not sharp.

DETD Other **proteins**

DETD Direct reduction of the **proteins** under study was determined by a modification of the method of Crawford, et al. (Crawford, N. A., et al. (1989),. . . to 70 .mu.l of the buffer solution containing 1 mM NADPH and 10 .mu.g (2 to 17 .mu.M) of target **protein**. When thioredoxin was reduced by dithiothreitol (DTT, 0.5 mM), NADPH and NTR were omitted. Assays with reduced glutathione were performed. . .

DETD Quantification of labeled **proteins**

DETD To obtain a quantitative indication of the extent of reduction of test **proteins** by the NADP/thioredoxin system, the intensities of their fluorescent bands seen in SDS-polyacrylamide gel electrophoresis were evaluated, using a modification. . . Ultrascan laser densitometer, and the area underneath the peaks was quantitated by comparison to a standard curve determined for each **protein**. For the latter determination, each **protein** (at concentrations ranging from 1 to 5 .mu.g) was reduced by heating for 3 min. at 100.degree. C. in the. . . and excess mBBr derivatized with .beta.-mercaptoethanol. Because the intensity of the fluorescent bands was proportional to the amounts of added **protein**, it was assumed that reduction was complete under the conditions used.

DETD . . . specific thioredoxin in the activation of chloroplast enzymes is one test for the ability of thiol groups of a given **protein** to undergo reversible redox change. Even though not physiological in the

case of extraplastidic **proteins**, this test has proved useful in several studies. A case in point is purothionin which, when reduced by thioredoxin h. . . . The FBPase, whose physiological activator is thioredoxin f, is unaffected by thioredoxin h. In this Example, the ability of cystine-rich **proteins** to activate FBPase as well as NADP-MDH was tested as set forth above. The .alpha.-amylase inhibitors from durum wheat (DSG-1. . .

DETD CM-1--the bread wheat **protein** that is similar to DSG **proteins** but has a lower molecular weight--also activated NADP-MDH and not FBPase when 20 .mu.g of CM-1 were used as shown. . . . that thioredoxin h reduces a variety of .alpha.-amylase inhibitors, which, in turn, activate NADP-MDH in accordance with equations 4-6. These **proteins** were ineffective in enzyme activation when DTT was added in the absence of thioredoxin. ##STR3##

DETD TABLE I

Effectiveness of Thioredoxin-Reduced Trypsin Inhibitors, Thionins, and .alpha.-Amylase Inhibitors in Activating Chloroplast NADP-Malate Dehydrogenase and Fructose Bisphosphatase (DTT.fwdarw.Thioredoxin.fwdarw.Indicated **Protein**.fwdarw.Target Enzyme)

Activation of NADPH--MDH was carried out as described above in this Example except that the quantity of DSG or the other **proteins** tested was 20 .mu.g. FBPase activation was tested using the standard DTT assay with 1 .mu.g of E. coli thioredoxin and 20 .mu.g of the indicated **proteins**. The above values are corrected for the limited activation seen with E. coli thioredoxin under these conditions.

*ACTIVITY, nkat/mg **Protein**	M.sub.r, kDa	No. of S--S Groups	NADP--MDH	FBPase
.alpha.-Amylase Inhibitors				
**DSG-2	17	5	2	0
**DSG-1	14	5	2	0
.dagger-dbl.CM-1	12	5	12	0
Trypsin Inhibitors				
Cystine-rich (plant)				
Corn. . .				

DETD . . . the reduction of the sulfhydryl reagent, 2',5'-dithiobis(2-nitrobenzoic acid) (DTNB), measured by an increase in absorbance at 412 nm. Here, the **protein** assayed was reduced with NADPH via NTR and a thioredoxin. The DTNB assay proved to be effective for the .alpha.-amylase. . . effective in the DTNB reduction assay, and, as with NADP-MDH activation (Table I), was detectably more active than the DSG **proteins** The conditions for the CM-1 assay were the same as for the DSG/DTNB assay except that the DSG **proteins** were omitted and purothionin .alpha., 20 .mu.g or CM-1, 20 .mu.g was used). The results thus confirmed the enzyme activation. . .

DETD **Protein** Reduction Measurements

DETD . . . and its adaptation for use in plant systems has given a new technique for measuring the sulfhydryl groups of plant **proteins** (Crawford, N. A., et al. (1989), Arch. Biochem. Biophys. 271:223-239). When coupled with SDS-polyacrylamide gel electrophoresis, mBBr can be used to quantitate the change in the sulfhydryl status of redox active **proteins**, even in complex mixtures. This technique was therefore applied to the inhibitor **proteins** to confirm their capacity for reduction by thioredoxin. Here, the test **protein** was reduced with thioredoxin which itself had been previously reduced with either DTT or NADPH and NTR. The mBBr derivative of the reduced **protein** was then prepared, separated from other components by

SDS-polyacrylamide gel electrophoresis and its reduction state was examined by fluorescence. In. . . experiments described below, thioredoxin from E. coli was found to be effective in the reduction of each of the targeted **proteins**. Parallel experiments revealed that thioredoxin h and calf thymus thioredoxins reduced, respectively, the **proteins** from seed and animal sources.

DETD . . . of the enzyme activation and dye reduction experiments, DSG-1 was effectively reduced in the presence of thioredoxin. Following incubation the **proteins** were derivatized with mBBr and fluorescence visualized after SDS-polyacrylamide gel electrophoresis. Reduction was much less with DTT alone and was. . .

DETD Whereas the major soluble cystine-rich **proteins** of wheat seeds can act as inhibitors of exogenous .alpha.-amylases, the cystine-rich **proteins** of most other seeds lack this activity, and, in certain cases, act as specific inhibitors of trypsin from animal sources. While these **proteins** can be reduced with strong chemical reductants such as sodium borohydride (Birk, Y. (1985), Int. J. Peptide **Protein** Res. 25:113-131, and Birl, Y. (1976), Meth. Enzymol. 45:695-7390), there is little evidence that they can be reduced under physiological. . .

DETD . . . inhibitors from seeds can undergo specific reduction by thioredoxin, the question arose as to whether other types of trypsin inhibitor **proteins** share this property. In the course of this study, several such inhibitors--soybean Kunitz, bovine lung aprotinin, egg white ovoinhibitor and ovomucoid trypsin inhibitors--were tested. While the parameters tested were not as extensive as with the cystine-rich **proteins** described above, it was found that the other trypsin inhibitors also showed a capacity to be reduced specifically by thioredoxin as measured by both the enzyme activation and mBBr/SDS-polyacrylamide gel electrophoresis methods. As was the case

for the cystine-rich **proteins** described above, the trypsin inhibitors tested in this phase of the study (soybean Kunitz and animal trypsin inhibitors) activated NADP-MDH. . . that it activated FBPase more effectively than NADP-MDH. It might also be noted that aprotinin resembles certain of the seed **proteins** studied here in that it shows a high content of cystine (ca. 10%) (Kassel, B., et al. (1965), Biochem. Biophys.. . .

DETD The fluorescence evidence for the thioredoxin-linked reduction of one of

these **proteins**, the Kunitz inhibitor, was shown by a highly fluorescent slow moving band in an mBBr/SDS-polyacrylamide electrophoretic gel. In its reduced. . .

DETD . . . ability to activate FBPase. The activity differences between these purothionins were unexpected in view of the strong similarity in their **amino acid** sequences (Jones, B. L., et al. (1977), Cereal Chem. 54:511-523) and in their ability to undergo reduction by thioredoxin. A. . .

DETD The above Examples demonstrate that thioredoxin reduces a variety of **proteins**, including .alpha.-amylase, such as the CM and DSG inhibitors, and trypsin inhibitors from seed as well as animal sources. While. . .

DETD As shown in Table II, the extent of reduction of the seed inhibitor **proteins** by the E. coli NADP/thioredoxin system was time-dependent and reached, depending on the **protein**, 15 to 48% reduction after two hours. The results, based on fluorescence emitted by the major **protein** component, indicate that thioredoxin acts catalytically in the reduction of the .alpha.-amylase and trypsin inhibitors. The ratio of **protein** reduced after two hours to thioredoxin added was greater than one for both the most highly

reduced **protein** (soybean Bowman-Birk trypsin inhibitor) and the least reduced **protein** (corn kernel trypsin inhibitor)--i.e., respective ratios of 7 and 2 after a two-hour reduction period. It should be noted that. . .

DETD TABLE II

Extent of Reduction of Seed **Proteins** by the NADP/Thioredoxin System Using the mBBr/SDS-Polyacrylamide Gel Electrophoresis Procedure

The following concentrations of **proteins** were used (nmoles): thioredoxin, 0.08; NTR, 0.01; purothionin-.beta., 1.7; DSG-1, 0.7; corn kernel trypsin inhibitor, 1.0; Bowman-Birk trypsin inhibitor, 1.3; and Kunitz trypsin inhibitor, 0.5. Except for the indicated time difference, other conditions were as in Examples 1-4.

Protein	% Reduction After 20 min	120 min
Purothionin-.beta.	15	32
DSG-1	22	38
Corn kernel trypsin inhibitor	3	15
Bowman-Birk trypsin inhibitor	25	48
Kunitz trypsin inhibitor	14	22

DETD Bacteria and animals are known to contain a thiol redox **protein**, glutaredoxin, that can replace thioredoxin in reactions such as ribonucleotide reduction (Holmgren, A. (1985), Annu. Rev. Biochem. 54:237-271). Glutaredoxin is reduced as shown in equations 10 and 11. ##STR5## So far there is no evidence that glutaredoxin interacts with **proteins** from higher plants. This ability was tested, using glutaredoxin from E. coli and the seed **proteins** currently under study. Reduction activity was monitored by the mBBr/SDS polyacrylamide gel electrophoresis procedure coupled with densitometric scanning. It was. . .

DETD The above Examples demonstrate that some of the enzyme inhibitor **proteins** tested can be reduced by glutaredoxin as well as thioredoxin. Those specific for thioredoxin include an .alpha.-amylase inhibitor (DSG-2), and several trypsin inhibitors (Kunitz, Bowman-Birk, aprotinin, and ovomucoid inhibitor). Those **proteins** that were reduced by either thioredoxin or glutaredoxin include the purothionins, two .alpha.-amylase inhibitors (DSG-1, CM-1), a cystine-rich trypsin inhibitor. . .

DETD . . . and FBPase target enzymes shown in Table I are low relative to those seen following activation by the physiological chloroplast **proteins** (thioredoxin m or f), the values shown were found repeatedly and therefore are considered to be real. It seems possible that the enzyme specificity shown by the inhibitor **proteins**, although not relevant physiologically, reflects a particular structure achieved on reduction. It remains to be seen whether such a reduced. . .

DETD . . . physiological consequence of the thioredoxin (or glutaredoxin) linked reduction event is of considerable interest as the function of the targeted **proteins** is unclear. The present results offer a new possibility. The finding that thioredoxin reduces a wide variety of inhibitor **proteins** under physiological conditions suggests that, in the absence of compartmental barriers, reduction can take place within the cell.

DETD . . . analysis of the ability of the treated flour for trypsin activity is made using modifications of the insulin and BAEE (Na-benzoyl-L-**arginine** ethyl ester) assays (Schoellmann, G., et al. (1963), Biochemistry 252:1963; Gonias, S. L., et al. (1983), J. Biol. Chem. 258:14682).. . .

DETD REDUCTION OF CEREAL **PROTEINS**

DETD For isolation of insoluble storage **proteins**, semolina (0.2 g)

was extracted sequentially with 1 ml of the following solutions for the indicated times at 25.degree. C.:. . .

DETD In vitro mBBr labelling of **proteins**

DETD . . . unless specified otherwise) were added to 70 .mu.l of this buffer containing 1 mM NADPH and 10 .mu.g of target **protein**. When thioredoxin was reduced by dithiothreitol (DTT), NADPH and NTR were omitted and DTT was added to 0.5 mM. Assays. . .

DETD In vivo mBBr labelling of **proteins**

DETD . . . a microfuge tube. The volume of the suspension was adjusted to 1 ml with the appropriate mBBr or buffer solution. **Protein** fractions of albumin/globulin, gliadin and glutenin were extracted from endosperm of germinated seedlings as described above. The extracted **protein** fractions were stored at -20.degree. C. until use. A buffer control was included for each time point.

DETD . . . Cereal Chem. 62:372-377). A gel solution in 100 ml final volume contained 6.0 g acrylamide, 0.3 g bisacrylamide, 0.024 g **ascorbic acid**, 0.2 mg ferrous sulfate heptahydrate and 0.25 g aluminum lactate. The pH was adjusted to 3.1 with lactic acid. The. . .

DETD . . . in 12% (w/v) trichloroacetic acid and soaked for 4 to 6 hr. with one change of solution to fix the **proteins**; gels were then transferred to a solution of 40% methanol/10% acetic acid for 8 to 10 hr. to remove excess mBBr. The fluorescence of mBBr, both free and **protein** bound, was visualized by placing gels on a light box fitted with an ultraviolet light source (365 nm). Following removal. . .

DETD **Protein** Staining/Destaining/Photography

DETD **Protein** stained gels were photographed with Polaroid type 55 film to produce prints and negatives. Prints were used to determine band. . .

DETD The Polaroid negatives of fluorescent gels and prints of wet **protein** stained gels were scanned with a laser densitometer (Pharmacia-LKB UltroScan XL). Fluorescence was quantified by evaluating peak areas after integration. . .

DETD **Protein** Determination

DETD **Protein** concentrations were determined by the Bradford method (Bradford, M. (1976) Anal. Biochem. 72:248-256), with Bio-Rad reagent and bovine serum albumin. . .

DETD As a result of the pioneering contributions of Osborne and coworkers a century ago, seed **proteins** can be fractionated on the basis of their solubility in aqueous and organic solvents (20). In the case of wheat, preparations of endosperm (flour or semolina) are historically sequentially extracted with four solutions to yield the indicated **protein** fraction: (i) water, albumins; (ii) salt water, globulins; (iii) ethanol/water, gliadins; and (iv) acetic acid/water, glutenins. A wide body of evidence has shown that different **proteins** are enriched in each fraction. For example, the albumin and globulin fractions contain numerous enzymes, and the gliadin and glutenin fractions are in the storage **proteins** required for germination.

DETD Examples 1, 4 and 5 above describe a number of water soluble seed **proteins** (albumins/globulins, e.g., .alpha.-amylase inhibitors, cystine-rich trypsin inhibitors, other trypsin inhibitors and thionines) that are reduced by the NADP/thioredoxin system, derived either from the seed itself or E. coli. The ability of the system to reduce insoluble storage **Proteins** from wheat seeds, viz., representatives of the gliadin and glutenin fractions, is described below. Following incubation with the indicated additions, the gliadin **proteins** were derivatized with mBBr and fluorescence was visualized after SDS-polyacrylamide gel electrophoresis. The lanes in this first gliadin gel were. . . NADPH, reduced glutathione, glutathione reductase (from

spinach leaves) and glutaredoxin (from E. coli). 4. NTS: NADPH, NTR, and thioredoxin (both **proteins** from E. coli). 5. MET/T(Ec): .beta.-mercaptoethanol and thioredoxin (E. coli). 6. DTT. 7. DTT/T(Ec): DTT and thioredoxin (E. coli). 8. DTT/T(W): Same as 7 except with wheat thioredoxin h. 9. NGS,-Gliadin: same as 3 except without the gliadin **protein** fraction. 10. NTS,-Gliadin: same as 4 except without the gliadin **protein** fraction. Based on its reactivity with mBBr, the gliadin fraction was extensively reduced by thioredoxin. The major members undergoing reduction. . . from 25 to 45 kDa. As seen in Examples 1, 4 and 5 with the seed .alpha.-amylase and trypsin inhibitor **proteins**, the gliadins were reduced by either native h or E. coli type thioredoxin (both homogeneous); NADPH (and NTR) or DTT could serve as the reductant for thioredoxin. Much less extensive reduction was observed with glutathione and glutaredoxin--a **protein** able to replace thioredoxin in certain E. coli and mammalian enzyme systems, but not known to occur in higher plants.

DETD The gliadin fraction is made up of four different **protein** types, designated .alpha., .beta., .gamma. and .omega., which can be separated by native polyacrylamide gel electrophoresis under acidic conditions (Bushuk,. . . (S--S) groups and thus has the potential for reduction by thioredoxin. In this study, following incubation with the indicated additions, **proteins** were derivatized with mBBr, and fluorescence was visualized after acidic-polyacrylamide gel electrophoresis. The lanes in the second gliadin gel in. . . leaves) and glutaredoxin (from E. coli). 5. NGS+NTS: combination of 4 and 6. 6. NTS: NADPH, NTR, and thioredoxin (both **proteins** from E. coli). 7. MET/T(Ec): .beta.-mercaptoethanol and thioredoxin (E. coli). 8. DTT/T(Ec): DTT and thioredoxin (E. coli). 9. NTS(-T): same. . .

DETD When the thioredoxin-reduced gliadin fraction was subjected to native gel electrophoresis, the **proteins** found to be most specifically reduced by thioredoxin were recovered in the a fraction. There was active reduction of the. . .

DETD The remaining group of seed **proteins** to be tested for a response to thioredoxin--the glutenins--while the least water soluble, are perhaps of greatest interest. The glutenins. . . and semolina (MacRitchie, F., et al. (1990), Adv. Cer. Sci. Tech. 10:79-145). Testing the capability of thioredoxin to reduce the **proteins** of this group was, therefore, a primary goal of the current investigation.

DETD . . . .alpha. .beta. .gamma. Aggregate*

None	22.4	30.4	24.3	29.2
Glutathione	36.4	68.1	60.6	60.1
Glutaredoxin	43.5	83.3	79.7	61.5
Thioredoxin	100.0	100.0	100.0	100.0

*__Proteins__ not entering the gel

DETD . . . glutaredoxin. However, in all cases, reduction was greater with thioredoxin and, in some cases, specific to thioredoxin (Table IV, note **proteins** in the 30-40 and 60-110 kDa range). As observed with the other wheat **proteins** tested, both the native h anal E. coli thioredoxins were active and could be reduced with either NADPH and the. . .

DETD . . . the wheat gliadin and glutenin fractions when tested in vitro. The results, however, provide no indication as to whether these **proteins** are reduced in vivo during germination--a question

that, to our knowledge, had not been previously addressed (Shutov, A. D., et. . .

DETD To answer this question, the mBBr/SDS-PAGE technique was applied to monitor the reduction status of **proteins** in the germinating seed. We observed that reduction of components in the Osborne fractions increased progressively with time and reached. . . to 3-fold with the

albumin/globulins and 5-fold with the glutenins. The results suggest that, while representatives of the major wheat **protein** groups were reduced during germination, the net redox change was greatest with the glutenins.

DETD Although providing new evidence that the seed storage **proteins** undergo reduction during germination, the results give no indication as to how reduction is accomplished, i.e., by glutathione or thioredoxin.. . . the reduction determined from in vitro measurements (cf. Table IV). For this purpose, the ratio of fluorescence to Coomassie stained **protein** observed in vivo during germination and in vitro with the appropriate enzyme reduction system was calculated. The results (principal thioredoxin. . .

DETD TABLE V

Activities of Enzymes Effecting the Reduction of Thioredoxin h in Semolina (Glucose.fwdarw.Glu-6-P.fwdarw.6-P-Gluconate.fwdarw.NADP.fwdarw.Thioredoxin h)

Protein	Activity (nkat/mg **protein**)
Hexokinase	0.28
Glucose-6-P dehydrogenase	0.45
6-P-Gluconate dehydrogenase	0.39
NTR	0.06
Thioredoxin h	0.35

DETD . . . pathway), thioredoxin h appears to function not only in the activation of enzymes, but also in the mobilization of storage **proteins**.

DETD Dough quality was improved by reducing the flour **proteins** using the NADP/thioredoxin system. Reduced thioredoxin specifically breaks sulfur-sulfur bonds that cross-link different parts of a **protein** and stabilize its folded shape. When these cross-links are cut the **protein** can unfold and link up with other **proteins** in bread, creating an interlocking lattice that forms the elastic network of dough. The dough rises because the network traps.

. . the gliadins and glutenins in flour letting them recombine in a way that strengthened the dough. Reduced thioredoxin strengthened the **protein** network formed during dough making. For these tests (using 10 gm of either intermediate quality wheat flour obtained from a.

. .

DETD . . . such as better crumb quality, improved texture and higher loaf volume. Also, based on in vivo analyses with the isolated **proteins**, the native wheat seed NADP/thioredoxin system will also be effective in strengthening the dough.

DETD . . . that yeast for purposes of leavening be added after the reduced

thioredoxin has had a chance to reduce the storage **proteins**. The dough is then treated as a regular dough proofed, shaped, etc. and baked.

DETD Reduction of Ethanol-Soluble and Myristate-Soluble Storage **Proteins** from Triticale, Rye, Barley, Oat, Rice, Sorghum, Corn and Teff

DETD . . . and methods used in this Example are according to those set

forth above in the section titled "Reduction of Cereal **Proteins**, Materials and Methods."

DETD . . . were added to 70 .mu.L of this buffer containing 1 mM NADPH and

25 to 30 .mu.g of extracted storage **protein**. The ethanol extracted storage **proteins** were obtained by using 50 ml of 70% ethanol for every 10 gm of flour and extracting for 2 hr. In the case of

teff, 200 mg of ground seeds were extracted. The myristate extracted **proteins** were obtained by extracting 1 gm of flour with 8 mg sodium myristate in 5 ml of distilled H.sub.2 O. . .

DETD The reactions were carried out in 30 mM Tris-HCl buffer, pH 7.9. When **proteins** were reduced by thioredoxin, the following were added to 70 .mu.L of buffer: 1.2 mM NADPH, 10 to 30 .mu.g of seed **protein** fraction, 0.5 .mu.g E. coli NTR and 1 ug E. coli thioredoxin. For reduction with glutathione, thioredoxin and NTR were. . . of 100 mM 2-mercaptoethanol were added and the samples applied to the gels. In each case, to obtain the extracted **protein**, 1 g ground seeds was extracted with 8 mg of sodium myristate in 5 ml distilled water. With the exception of the initial redox state determination of the **proteins**, samples were extracted for 2 hr at 22.degree. C. and then centrifuged 20 min at 16,000 rpm prior to the.

. .

DETD Separate SDSS-polyacrylamide electrophoretic gels of the reduction studies of myristate-extracted **proteins** from flour of oat, triticale, rye, barley and teff were prepared. A gel showing the extent of thioredoxin linked buffer and ethanol-extracted **proteins** for teff was also prepared. In all of the oat, triticale, rye, barley, teff/myristate extractions studies, the flour was first. . . 7.5 for 20 min. and then with 70% ethanol for 2 hr. In addition, gels were prepared for the myristate-extracted **proteins** from corn, sorghum and rice. With corn, sorghum and rice, the ground seeds were extracted only with myristate. Therefore, with corn, sorghum and rice, the myristate extract represents total **protein**, whereas with oat, triticale, rye, barley and teff, the myristate extract represents only the glutenin-equivalent fractions since these flours had. . . depicted in the gels, show that the NTS is most effective, as compared to GSH or GSH/GR/NADPH, with myristate-extracted (glutenin-equivalent) **proteins** from oat, triticale, rye, barley and teff. The NTS is also most effective with the total **proteins** from rice. Reduced glutathione is more effective with the total **proteins** from corn and sorghum.

DETD In the first gel relating to the effect of NTS vs. glutathione reductase

on the reduction status of the myristate-extracted **proteins**, in treatment (1), extraction with myristate in the presence of mBBr was carried out under a nitrogen atmosphere; in treatment (2), to the myristate extracted **proteins** mBBr was added without prior reduction of the **proteins**; in treatment (3), the myristate extracted **proteins** were reduced by the NADP/thioredoxin system (NTS); in treatment (4) the myristate extracted **proteins** were reduced by NADPH, glutathione and glutathione reductase. In the second gel relating to the in vivo reduction status and thioredoxin linked in vitro reduction of the myristate-extracted **proteins**, treatment (1) is like treatment (2) in the first gel; in treatment (2) the seeds were extracted with myristate in. . . and reduced by the NTS and then

mBBr was added; and in treatment (4) conditions as in (3) except that **proteins** were reduced by DTT. Treatment (1) in the first gel and treatment (2) in the second gel showed the initial redox state of the **proteins** in the grains. For all three cereals, the **proteins** in the seed were highly reduced. If extracted in air, the **proteins** became oxidized especially the sorghum and rice. The oxidized **proteins** can be re-reduced, maximally with NTS in all cases. With rice, the reduction was relatively specific for

thioredoxin; with corn,. . . glutathione is slightly more effective than thioredoxin. Dithiothretol showed varying effectiveness as a reductant. These experiments demonstrated that the storage **proteins** of these cereals are less specific than in the case of wheat and suggest that thioredoxin should be tested both. . .

DETD . . . hr. The gels showed that the yeast system is highly active in reducing the two major groups of wheat storage **proteins**.

DETD Gels for the reduction of ethanol-extracted **proteins** from flour of triticale, rye, oat and barley, respectively, were also prepared. The results showed that the NTS is most effective with the ethanol-extracted **proteins** from triticale, rye and oat. The ethanol-extracted barley **proteins** were reduced in the control and thioredoxin or glutathione had little effect.

DETD Effect of Thioredoxin-linked Reduction on the Activity and Stability of the Kunitz and Bowman-Birk Soybean Trypsin Inhibitor **Proteins**

DETD . . . from Sigma Chemical Co. (St. Louis, Mo.). E. Coli thioredoxin and NTR were isolated from cells transformed to overexpress each **protein**. The thioredoxin strain containing the recombinant plasmid, pFPI, was kindly provided by Dr. J.-P. Jacquot (de La Motte-Guery et al.,. . . was kindly provided by Drs. Marjorie Russel and Peter Model (Russel and Model, 1988). The isolation procedures used for these **proteins** were as described in those studies with the following changes: cells were broken in a Ribi cell fractionator at 25,000. . .

DETD . . . slab gels were scanned with a laser densitometer (Pharmacia-LKB UltraScan XL) and the peak area of the KTI or BBTI **protein** band was obtained by integration with a Pharmacia GelScan XL software program.

DETD . . . Trypsin activity was measured in 50 mM Tris-HCl, pH 7.9, by following the increase in absorbance at 253 nm with N-benzoyl-L-**arginine** ethyl ester as substrate (Mundy et al., 1984) or by the release of azo dye into the trichloroacetic acid (TCA)-soluble. . .

DETD . . . supernatant solution was withdrawn and mixed with 1 ml of 1 N NaOH. The absorbance was read at 440 nm. **Protein** concentration was determined with Bio-Rad reagent using bovine serum albumin as a standard (Bradford, 1976).

DETD . . . specifically by the NADP/thioredoxin system from either E. coli or plants. The reduced forms of glutathione and glutaredoxin (a thiol **protein** capable of replacing thioredoxin in certain animal and bacterial systems, but not known to occur in plants (Holmgren, 1985)) were. . .

DETD . . . 88.9

Reduced by LA/Trx h.sup.3

40.5 87.8

---

*The specific activity of the uninhibited control trypsin was 0.018 .DELTA.A.sub.253 nm /.mu.g/min using Nbenzoyl-L-**arginine** ethyl ester as substrate.

.sup.1 Reduction by E. coli NTS (NADP/thioredoxin system) was conducted a 30.degree. C. for 2 hours.

. . .

DETD Friedman and colleagues observed that heating soybean flour in the presence of sulfur reductants (sodium sulfite, N-acetyl-L-**cysteine**, reduced glutathione, or L-**cysteine**) inactivated trypsin inhibitors, presumably as a result of the reduction or interchange of disulfide groups with other **proteins** in soy flour (Friedman and Gumbmann, 1986; Friedman et al., 1982, 1984). Inactivation of the trypsin inhibitors by these reductants. . .

DETD Protease inhibitor **proteins** are typically stable to inactivation treatments such as heat. This stability is attributed, at least in part, to the cross-linking. . .

DETD . . . and the proteolytic products were analyzed by SDS-PAGE. The extent of proteolysis was determined by measuring the abundance of

intact **protein** on SDS gels by laser densitometer. When tested with a protease preparation from 5-day germinated wheat seeds, the oxidized form. . . reaction that depended on all components of the NADP/thioredoxin system (NTS). BBTI showed the same pattern except that the oxidized **protein** showed greater proteolytic susceptibility relative to KTI. Similar effects were observed with both inhibitors when the plant protease preparation was. . .

DETD This Example shows that reduction by thioredoxin, or dithiothreitol (DTT), leads to inactivation of both **proteins** and to an increase in their heat and protease susceptibility. The results indicate that thioredoxin-linked reduction of the inhibitor **proteins** is relevant both to their industrial processing and to seed germination. These results confirm the conclusion that disulfide bonds are. . .

DETD . . . exposed to the protease inhibitors during seed germination, the NADP/thioredoxin system could serve as a mechanism by which the inhibitor **proteins** are modified (inactivated) and eventually degraded (Baumgartner and Chrispeels, 1976; Chrispeels and Baumgartner, 1978; Orf et al., 1977; Wilson, 1988;. . . Yoshikawa et al., 1979). As stated previously, there is evidence that the NADP-thioredoxin system plays a similar role in mobilizing **proteins** during the germination of wheat seeds.

DETD Reduction of Castor Seed 2S Albumin **Protein** by Thioredoxin

DETD The results of the following study of sulfhydryl agents to reduce the 2S **protein** from castor seed (Sharief and Li, 1982; Youle and Huang, 1978) shows that thioredoxin actively reduces intramolecular disulfides of the. . .

DETD . . . from Sigma Chemical Co. (St. Louis, Mo.). E. Coli thioredoxin and NTR were isolated from cells transformed to overexpress each **protein**. The thioredoxin strain containing the recombinant plasmid pFPI, was kindly provided by Dr. J.-P. Jacquot (de La Mott-Guery et al.. . . (Nishizawa et al. 1982), respectively. Thioredoxin h was isolated from wheat seeds by following the procedure devised for the spinach **protein** (Florencio et al. 1988). Glutathione reductase was prepared from spinach leaves (Florencio et al. 1988).

DETD Isolation of **Protein** Bodies

DETD **Protein** bodies were isolated by a nonaqueous method (Yatsu and Jacks, 1968). Shelled dry castor seeds, 15 g, were blended with. . . a JS-20 rotor. After centrifugation, the supernatant fraction was collected and centrifuged 20 min at 41,400.times.g. The pellet, containing the **protein** bodies, was resuspended in 10 ml glycerol and centrifuged as before (41,400.times.g for 20 min) collecting the pellet. This washing. . .

DETD 2S **Protein** Purification Procedure

DETD The 2S **protein** was prepared by a modification of the method of Tully and Beevers (1976). The matrix **protein** fraction was applied to a DEAE-cellulose (DE-52) column equilibrated with 5 mM Tris-HCl buffer, pH 8.5 (Buffer A) and eluted with a 0 to 300 mM NaCl gradient in buffer A. Fractions containing the 2S **protein** were pooled and concentrated by freeze drying. The concentrated fraction was applied to a Pharmacia FPLC Superose-12 (HR 10/30) column equilibrated with buffer A containing 150 mM NaCl. The fraction containing 2S **protein** from the Superose-12 column was applied to an FPLC Mono Q HR 5/5 column equilibrated with buffer A. The column. . . gradient of 0 to 300 mM NaCl in buffer A and finally with buffer A containing 1M NaCl. The 2S **protein** purified by this method was free of contaminants in SDS polyacrylamide gels stained with Coomassie blue (Kobrehel et al., 1991).

DETD Reduction of **proteins** was monitored by the monobromobimane (mBBr)/SDS polyacrylamide gel electrophoresis procedure of Crawford et al. (1989). Labeled **proteins** were quantified as described

previously in the "Reduction of Cereal **Proteins**, Materials and Methods" section. **Protein** was determined by the method of Bradford (1976).

DETD . . . al., 1981 protocol was used for assaying NADP-malate dehydrogenase and fructose 1,6 bisphosphatase in the presence of thioredoxin and 2S **protein**. Assays were conducted under conditions in which the amount of added thioredoxin was sufficient to reduce the castor 2S **protein** but insufficient to activate the target enzyme appreciably. All assays were at 25.degree. C. Unless otherwise indicated, the thioredoxin and NTR used were from E. coli.

The 2S **protein** was monitored during purification by mBBr/SDS-polyacrylamide gel electrophoresis following its reduction by dithiothreitol and E. coli thioredoxin (Crawford et al.,. . .

DETD The reduction of the matrix and crystalloid **proteins** from castor seed were determined by the mBBr/SDS-polyacrylamide gel electrophoresis procedure. The lanes for the gels (not shown) were as. . . glutathione, glutathione reductase (from spinach leaves) and glutaredoxin from E. coli; 4 and 10, NTS: NADPH, NTR, and thioredoxin (both **proteins** from E. coli); 5 and 11, NADPH; 6 and 12, NADPH and E. coli NTR. Reactions were carried out in. . . .mu.g NTR and 1 .mu.g thioredoxin were added to 70 .mu.l of this buffer containing 1 mM NADPH and target **protein**: 8 .mu.g matrix **protein** for treatments 1-6 and 10 .mu.g crystalloid **protein** for treatments 7-12. Assays with glutathione were performed similarly, but at a final concentration of 2 mM, 1.4 .mu.g glutathione. . .

DETD . . . mBBr/SDS-Page technique was also used to determine the specificity of thioredoxin for reducing the disulfide bonds of castor seed 2S **protein**. The lanes for the gel (not shown) were as follows, (1) Control (no addition); (2) Control+NTS (same conditions as with the matrix and crystalloid **proteins**); (3) Control (heated 3 min at 100.degree. C.); (4) Control+2 mM DTT (heated 3 min at 100.degree. C.). The samples containing 5 .mu.g 2S **protein** and the indicated additions were incubated for 20 min in 30 mM Tris-HCl (pH 7.8). mBBr, 80 nmol, was then. . .

DETD The castor storage **proteins**, which are retained within a **protein** body during seed maturation, can be separated into two fractions on the basis of their solubility. The more soluble **proteins** are housed in the **protein** body outer section ("matrix") and the less soluble in the inner ("crystalloid"). In the current study, the matrix and crystalloid. . . isolated to determine their ability to undergo reduction by cellular thiols, viz., glutathione, glutaredoxin and thioredoxin. Glutaredoxin, a 12 kDa **protein** with a catalytically active thiol group, can replace thioredoxin in certain enzymic reactions of bacteria and animals (Holmgren et al.. . .

DETD The results showed that, while a number of storage **proteins** of castor seed were reduced by the thiols tested, only a low molecular weight **protein**, corresponding to the large subunit of the 2S **protein** of the matrix, showed strict specificity for thioredoxin. Certain higher molecular weight **proteins** of the crystalloid fraction underwent reduction, but in those cases there was little difference between glutaredoxin and thioredoxin. The castor seed 2S large subunit thus appeared to resemble cystine-containing **proteins** previously discussed in undergoing thioredoxin-specific reduction. These experiments were designed to confirm this specificity and to elucidate certain properties of the reduced **protein**. As expected, owing to lack of disulfide groups, the 2S small subunit showed essentially no reaction with mBBr with any. . .

DETD . . . found to depend on all components of the NADP/thioredoxin system (NADPH, NTR and thioredoxin) (Table XIV). As for other thioredoxin-linked **proteins** (including chloroplast enzymes), the thioredoxin active in reduction of the 2S large subunit could be reduced either chemically with dithiothreitol. . . 67% and 90%, respectively, after 20 min at 25.degree. C. Similar, though generally

extensive reduction was observed with the disulfide **proteins** discussed above (Johnson et al. 1987). As with the other seed **proteins**, native wheat thioredoxin h and E. coli thioredoxins could be used interchangeably in the reduction of the 2S **protein** by DTT (data not shown).

DETD TABLE XIV

Extent of reduction of the castor castor seed 2S **protein** by different sulfhydryl reductants. Reaction conditions as with the matrix and crytalloid **protein** determination. A reduction of 100% corresponds to that obtained when the 2S **protein** was heated for 3 min in the presence of 2% SDS and 2.5% .beta.-mercaptoethanol. NTS: NADPH, NTR, and thioredoxin (both **proteins** from E. coli); GSH/GR/NADPH: reduced glutathione, glutathione reductase (from spinach leaves) and NADPH; NGS: NADPH, reduced glutathione, glutathione reductase (from spinach leaves) and glutaredoxin. . .

DETD The capability of thioredoxin to reduce the castor seed 2S **protein** was also evident in enzyme activation assays. Here, the **protein** targeted by thioredoxin (in this case 2S) is used to activate a thioredoxin-linked enzyme of chloroplasts, NADP-malate dehydrogenase or fructose 1,6-bisphosphatase. As with most of the **proteins** examined so far, the 2S **protein** more effectively activated NADP-malate dehydrogenase and showed little activity with the fructose bisphosphatase (2.6 vs. 0.0 nmoles/min/mg **protein**).

DETD The castor seed 2S **protein** contains inter-as well as intramolecular disulfides. The 2S **protein** thus provides an opportunity to determine the specificity of thioredoxin for these two types of bonds. To this end, the castor seed 2S **protein** was reduced (i) enzymically with the NADP/thioredoxin system at room temperature, and (ii) chemically with DTT at 100.degree. C. Following reaction with mBBr the reduced **proteins** were analyzed by SDS-polyacrylamide gel electrophoresis carried out without additional sulfhydryl agent. The results indicate that while thioredoxin actively reduced. . .

DETD The present results extend the role of thioredoxin to the reduction of the 2S **protein** of castor seed, an oil producing plant. Thioredoxin specifically reduced the intramolecular disulfides of the large subunit of the 2S **protein** and showed little activity for the intermolecular disulfides joining the large and small subunits. Based on the results with the. . . trypsin inhibitors of soybean, it is clear that reduction of intramolecular disulfides by thioredoxin markedly increases the susceptibility of disulfide **proteins** to proteolysis (Jiao et al. 1992a). It, however, remains to be seen whether reduction of the 2S **protein** takes place prior to its proteolytic degradation (Youle and Huang, 1978) as appears to be the case for the major storage **proteins** of wheat. A related question raised by this work is whether the 2S **protein** of castor, as well as other oil producing plants such as brazil nut (Altenbach et al., 1987; Ampe et al., 1986), has a function in addition to that of a storage **protein**.

DETD Thioredoxin-Dependent Deinhibition of Pullulanase of Cereals by Inactivation of a Specific Inhibitor **Protein**

DETD . . . at 30,000 g and at 4.degree. C. for 25 min, the supernatant was fractionated by precipitation with solid ammonium sulfate. **Proteins** precipitated between 30% and 60% saturated ammonium sulfate were dissolved in a minimum volume of 20 mM Tris HCl, pH. . .

DETD . . . centrifuged to remove insoluble materials and the supernatant adjusted to pH 4.6 with 2N formic acid. After pelleting the acid-denatured **protein**, the supernatant was readjusted to pH 7.5 with NH.sub.4 OH and loaded onto a DE52 column (2.5.times.26 cm)

equilibrated with. . . 4.6) and Sephacryl-200 HR (30 mM Tris-HCl, pH 7.5, containing 200 mM NaCl and 1 mM EDTA) chromatography. Pullulanase inhibitor **protein** was purified as described below.

DETD . . . centrifugation and the supernatant was chromatographed on a CM32 column (2.5.times.6 cm) equilibrated with 20 mM sodium acetate, pH 4.6. **Proteins** were eluted with a linear 0-0.4M NaCl in 200 ml of 20 mM sodium acetate, pH 4.6. Fractions (5.0 ml/fraction). . .

DETD . . . conducted for the regulation of amylases, little is known about the regulation of pullulanase in seeds. Yamada (Yamada, J. (1981) **Carbohydrate** Research 90:153-157) reported that incubation of cereal flours with reductants (e.g., DTT) or proteases (e.g., trypsin) led to an activation. . . that is precipitated by ammonium sulfate and inhibits pullulanase. The role of DTT is to reduce and thus inactivate this **protein** inhibitor, leading to activation of pullulanase. Along this line, the 30-60% ammonium sulfate fraction from barley malt was applied to. . . with 20 mM Tris-CHl, pH 7.5. Following elution with a linear salt gradient, "deinhibited" ("activated") pullulanase was identified as a **protein** peak coming off at about 325 mM NaCl (from fraction numbers 44 to 60). Assay of pullulanase activity in the. . . preincubation mixture consisting of 50 .mu.l of the peak pullulanase activity fraction (fraction number 45) with 50 .mu.l of other **protein** fracitons indicated that a **protein** peak that showed pullulanase inhibitory activity was eluted from the DE52 column by about 100 mM NaCl between fraction numbers. . .

DETD Preliminary experiments showed that pullulanase inhibitor **protein** is resistant to treatment of 70.degree. C. for 10 min and pH 4.0. Based on the profile of Sephadex G-75. . . SDS-PAGE, pullulanase inhibitor has a molecular weight between 8 to 15 kDa .+-.2 kDa. The study further showed that the **protein** contains thioredoxin-reducible (S--S) bonds.

DETD These studies, as shown in Table XV, found that the ubiquitous dithiol **protein**, thioredoxin, serves as a specific reductant for a previously unknown disulfide-containing **protein** that inhibits pullulanase of barley and wheat endosperm.

DETD TABLE XV

Activity Change in Pullulanase Inhibitor **Protein** Following Reduction by NADP/Thioredoxin System

Treatment	Relative Pullulanase Activity
No inhibitor	100
Inhibitor	
Oxidized	30.1
Reduced by DTT	46.1
Reduced by E. coli Trx/DTT	95.1
Reduced by E.. . .	

DETD Reduction of the inhibitor **protein** eliminated its ability to inhibit pullulanase, thereby rendering the pullulanase enzyme active. These studies as shown in Table XV illustrate. . . several sources such as E. coli or seed endosperm (thioredoxin h). The role of thioredoxin in reductively inactivating the inhibitor **protein** (I) and deinhibiting the pullulanase enzyme (E) is given in Equations 1 and 2. ##STR6##

DETD In summary, the crude endosperm extracts were fractionated by column chromatography procedures. These steps served to separate the **protein** inhibitor from the pululanase enzyme. The inhibitor **protein** was then highly purified by several steps. By use of the mBBr/SDS-PAGE procedure, it was determined that disulfide group(s) of the new **protein** are specifically reduced by thioredoxin and that the reduced **protein** loses its ability to inhibit pullulanase. Like certain other disulfide **proteins** of seeds

(e.g., the Kunitz and Bowman-Birk trypsin inhibitors of soybean), the pullulanase inhibitor **protein** showed the capability to activate chloroplast NADP-malate dehydrogenase. In these experiments, dithiothreitol was used to reduce thioredoxin, which in turn. . .

DETD . . . amino terminus of the pure reductase enzyme is determined by microsequencing by automated Exman degradation with an Applied Biosystems gas-phase **protein** sequencer. On the basis of this sequence, and relying on codon usage in yeast, a 20-base 24-bold degenerate DNA probe. . .

DETD . . . its technological value: (1) by obtaining stronger glutens (increased elasticity, improved extensibility); (2) by increasing gluten yield by capturing soluble **proteins**, reduced by the NADP-thioredoxin system, in the **protein** network, thereby preventing them from being washed out during the production of gluten. In this procedure (using 10 g flour),. . .

DETD The invention provides a method for chemically reducing toxicity causing **proteins** contained in bee, scorpion and snake venome and thereby altering the biological activity of the venoms well as reducing the. . .

DETD . . . the reduced or sulfhydryl (SH) state. As defined herein the term "thiol redox (SH) agent" means a reduced thiol redox **protein** or synthetically prepared agent such as DTT.

DETD . . . Sigma Chemical Co. (St. Louis, Mo.). As the phospholipase A.sub.2 was provided in 3.2M (NH.sub.4).sub.2 SO.sub.4 solution pH 5.5, the **protein** was dialysed in 30 mM Tris-HCl buffer, pH 7.9, using a centricon 3 KDa cutoff membrane. .alpha.-Bungarotoxin and .alpha.-bungarotoxin.sup.125 I. . .

DETD Reagents and Fine chemicals DL-.**alpha**.-**Lipoic acid**, L-.alpha.-phosphatidylcholine from soybean, NADPH and .beta.-mercaptoethanol were purchased from Sigma Chemical Co. (St Louis, Mo.) and monobromobimane (mBBr, trade name. . .

DETD **Proteins** and Enzymes

DETD . . . to a solution containing 40% methanol and 10% acetic acid for 12 hr to remove excess mBBr. The fluorescence of **protein**-bound mBBr was determined by placing gels on a light box fitted with an ultraviolet light source (365 nm). Gels were. . . through a yellow Wratten gelatin filter No. 8 (cutoff=460 nm) (exposure time 40 sec. at f4.5). Gels were stained for **protein** for 1 hr in solution of 0.125% (w/v) Coomassie blue R-250 in 10% acetic acid and 40% methanol. Gels were. . .

DETD . . . were boiled for 3 min, and then subjected to SDS-polyacrylamide gel electrophoresis. Gels were stained with Coomassie blue and the **protein** bands quantified by densitometric scanning as described above. The results of the assay are shown in Table XVI below. These. . .

DETD The invention provides a method for chemically reducing the disulfide bonds in major food allergen **proteins** and for decreasing or eliminating the allergenicity that occurs when foods containing those **proteins** are ingested. The disulfide bonds are reduced to the sulfhydryl (SH) level by thioredoxin. The other major cellular thiol reductant, glutathione, was inactive in this capacity. The **proteins** are allergenically active in the oxidized (S--S) state; when treated with thioredoxin (SH state), they lose allergenicity. Thioredoxin achieves this. . . by the food depends upon the ability of the reduced thioredoxin to reduce the intramolecular disulfide bonds in the allergenic **proteins** in the food.

DETD Food allergen **proteins** that have intramolecular disulfide bonds can be reduced and the allergenicity of foods containing these **proteins** can be at least decreased by reduced thioredoxin when the food is treated with the thioredoxin for an effective period. . . about 20 min to 2 hrs, more preferably from about 30 min to 1 hr and 30 min. However, the **proteins** may also be reduced, for example,

by incubation at 5.degree. C. for 48 hrs.

DETD . . . about 1.5 micromoles to 30 micromoles, preferably about 5 micromoles to 20 micromoles, of NADPH for every 25 gm of **protein** in the food.

DETD . . . the thioredoxin and NTR used were purified as previously described from E. coli that had been transformed to overproduce those **proteins** (de la Motte-Guery, F. et al. (1991) Eur. J. Biochem. 196:287-294, and Russel, M. et al. (1988) J. Biol. Chem.. . .

DETD . . . samples were analyzed by the mBBr/SDS-polyacrylamide gel electrophoresis technique previously described. The results showed that the NTS effectively reduced the **proteins** in the allergenic extracts at both room temperature and 37.degree. C. In an additional study, where a PBS dilution of. . . was similarly treated with the NTS, an analysis using the mBBr labeling/SDS-PAGE method showed that thioredoxin also reduced the soy **proteins**. However, when soy, cow's milk, wheat, egg and beef allergenic **proteins** were incubated with glutathione, glutathione reductase and NADPH, there was minimal or no reduction of those treated allergenic **proteins**.

DETD . . . also similarly incubated with the NTS and analyzed using the mBBr/SDS-PAGE technique to show that reduced thioredoxin reduces rice allergen **proteins**.

DETD In a separate study, it was also observed that food allergen **proteins** from the commercial extracts described in Example 33 that had been reduced by the NTS and were further incubated with. . . was done using the mBBr/SDS-PAGE techniques. Further in this study, when

10 .mu.g of an NTS reduced purified milk allergen **protein**, .beta.-lactoglobulin (Sigma Chemical Co.), was treated with 2 .mu.g of trypsin, proteolysis was 100% as compared with only 50% for. . .

DETD Reduction of Egg White **Proteins**

DETD Dried chicken egg white was purchased from Sigma Chemical Co. About 80% of the total **proteins** in egg white are allergens. A solution of 20 mg/ml egg white was resuspended in PBS. Since not all the material

was dissolved, it was centrifuged at 14,000 RPM for 2 min. The soluble egg white **proteins** were used for the reduction study using mBBr fluorescent labelling and SDS-polyacrylamide gel electrophoresis analysis. The treatments used were the. . . thioredoxin, reduced glutathione (GSH) and reduced glutathione/glutathione reductase/NADPH. Reactions were carried out in PBS with 23 microliters of the soluble **proteins** from the 20 mg/ml egg white suspension in a final volume of 100 microliters. In the NTS, 7.5 mM NADPH,. . . The results

of this experiment showed that the NTS and DTT plus thioredoxin are very

effective in reducing egg white **proteins** which are about 80% allergens. GSH or GSH/GR/NADPH showed the same level of reduction as the

control and therefore is an ineffective reductant of egg white **proteins**.

DETD . . . were nursed for 6 weeks and weaned onto regular Puppy Chow (Ralston-Purina Company, St. Louis, Mo.) which included the sensitizing **proteins**, soybean meal, dried whey, and rice hulls; they were fed once/day and given water ad lib, under veterinary care and. . .

DETD . . . litter were given double-blinded, 240 ml of either soy or cow's

milk infant formula, tofu, rice gruel, or vanilla-flavored Vivonex **protein** hydrolysate (Norwich-Eaton Co., Norwich, Conn.) in the early morning. Abdominal girth was measured at umbilical level before the challenge and. . .

DETD Canine IgE-RASTs to the 3 food **proteins** were followed at fortnightly intervals with venous blood sampling.

DETD . . . in the same amount that they received as neonates. As above, they were fed foods which contained the appropriate sensitizing **proteins** (i.e., cow's milk, soy, beef and wheat) in a similar schedule. As with the previous soy and milk allergic animals,. . .

DETD . . . the 3 dogs of the assigned group. The portions fed to the animals were equivalent to 25.0 gm of soy **protein** prior to incubation.

DETD . . . and fed to the 3 dogs of the assigned lot. Again these portions were equivalent to 25 gm of wheat **protein**. In the experiment with 8 dogs, the flour was increased to 2.0 kg and the procedure scaled up accordingly.

DETD . . . and 3 received the treated milk. The final portions that the dogs received were equivalent to 10 gm of milk **protein**.

DETD . . . 15. In this feeding study, preliminary trials indicated that higher levels of these compounds were required to reduce the allergenic **proteins** as determined in vitro by the mBBr/SDS-polyacrylamide gel procedure. The amounts of each component of the NADP/thioredoxin system used for each dog per gm **protein** in the feeding trials is indicated below relative to the amounts used in the baking tests:

DETD . . . baking tests in which 3 micrograms thioredoxin, 1.5 micrograms NTR and 0.

micromoles NADPH were added per gram of flour **protein**. In the baking tests, loaves were baked with approximately 200 g flour or approximately 20 gm of flour **protein**. For the feeding experiments, food preparations were incubated with components of the NADP/thioredoxin system for one hour either at room. . .

DETD . . . or decreasing the allergenicity of several foods, namely wheat, egg, milk, soy and beef. The invention further provides a novel **protein** that is a pullulanase inhibitor and a method for its inactivation.

CLM What is claimed is:

. . . the NTR is at least 100 .mu.g and the added NADPH is at least 5 micromoles per 25 grams of **protein** in said food product.

. . . is about 100 .mu.g to 200 .mu.g and NADPH is about 5 micromoles to 20 micromoles per 25 grams of **protein** in said product.

6. The food product of claim 3 wherein the product contains beef, egg, soy, wheat or milk **protein**.

8. The food product of claim 7 wherein the added thioredoxin is at least 200 .mu.g, the NTR is at least 100 .mu.g and the added NADPH is at least 5 micromoles per 25 grams of **protein** in said food product.

. . . the NTR is at least 100 .mu.g and the added NADPH is at least 5 micromoles per 25 grams of **protein** in said food product.

. . . the NTR is at least 100 .mu.g and the added NADPH is at least 5 micromoles per 25 grams of **protein** in said food product.

. . . the NTR is at least 100 .mu.g and the added NADPH is at least 5 micromoles per 25 grams of **protein** in said food product.

. . . the NTR is at least 100 .mu.g and the added NADPH is at least 5 micromoles per 25 grams of **protein** in said food product.

. . . the NTR is at least 100 .mu.g and the added NADPH is at least 5 micromoles per 25 grams of **protein** in said food product.

AN 1998:95283 USPATFULL|

TI Neutralization of food allergens by thioredoxin|

IN Buchanan, Bob B., Berkeley, CA, United States
Kobrehel, Karoly, Montpellier, France
Yee, Boihon C., Walnut Creek, CA, United States

Lozano, Rosa, Madrid, Spain
Frick, Oscar L., San Francisco, CA, United States
Ermel, Richard W., Winters, CA, United States
PA The Regents of the University of California, Oakland, CA, United States (U.S. corporation)
PI US 5792506 19980811 <--
AI US 1994-326976 19941021 (8)
RLI Continuation-in-part of Ser. No. US 1994-211673, filed on 12 Apr 1994 which is a continuation-in-part of Ser. No. US 1992-935002, filed on 25 Aug 1992, now abandoned which is a continuation-in-part of Ser. No. US 1991-776109, filed on 12 Oct 1991, now abandoned
DT Utility|
EXNAM Primary Examiner: Grimes, Eric|
LREP Smith, Karen S.Flehr Hohbach Test Albritton & Herbert LLP|
CLMN Number of Claims: 25|
ECL Exemplary Claim: 1|
DRWN 6 Drawing Figure(s); 6 Drawing Page(s)|
LN.CNT 3602|
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 56 OF 82 USPATFULL
PI US 5747545 19980505 <--
SUMM This invention relates to the treatment of nervous system disorders, particularly disorders mediated by the N-methyl-D-aspartate (NMDA) subtype of excitatory **amino acid** receptor complex.
SUMM . . . conditions, the patient could be treated prophylactically according to the invention. Other diseases mediated (at least in part) by excitatory **amino acid** toxicity and can be treated by NMDA receptor complex modulation according to the present invention. Such diseases include: 1) ALS. . .
SUMM . . . any nucleophile including an amine; and agents which generate an oxidizing cascade similar to that generated by N.sup.2 such as . **alpha.-lipoic acid** (thioctic acid and its enantiomers); dihydrolipoate; glutathione; ascorbate; and vitamin E.
SUMM . . . used to enhance absorption into the central nervous system (CNS) and efficacy of SOD and/or catalase. An SOD mimic, the **protein**-bound polysaccharide of Coriolus versicolor QUEL, termed "PS-K", may also be effective by parenteral or oral routes of administration, especially with. . .
DRWD . . . to form an RS-NO (NO.sup.+ equivalent). This chemical reaction leads to a decrease in NMDA receptor-operated channel activation by excitatory **amino acids** (such as NMDA or glutamate) and a concomitant decrease in intracellular calcium influx and amelioration of neurotoxicity.
DRWD . . . above). In addition to glutamate itself, neuronal injury may result from stimulation of the NMDA receptor-channel complex by other excitatory **amino acids**, such as aspartate, quinolinate, homocysteic acid, **cysteine** sulfonic acid, **cysteine**, or from stimulation by excitatory peptides, such as N-acetyl aspartyl glutamate.
DRWD . . . receptor complex-mediated injury, e.g., that injury resulting from stimulation of the NMDA receptor by NMDA(as shown below) or other excitatory **amino acids** or stimulation by excitatory peptides, such as N-acetyl aspartyl glutamate.
DETD . . . experiment of Example 3 was repeated using 1-5 mM NEM, N-ethylmalemide, an agent known to alkylate sulfhydryl (thiol) groups of **proteins**. Following alkylation, neither NTG nor DTNB significantly affected the amplitude of NMDA evoked current, indicating that the redox modulatory site. . .
DETD S-nitrosocysteine (SNOC) both liberates NO.sup..cndot. and participates in nitrosation (NO.sup.+ equivalents reacting with **protein** thiol groups). FIG. 5A is a digital representation of fura-2 calcium images as described above, for 10 cortical neurons in. . .

DETD . . . to enhance their absorption into the CNS and efficacy (Liu et al.,(1989) Am. J. Physiol. 256:589-593. An SOD mimic, the **protein**-bound polysaccharide of Coriolus versicolor QUEL, termed "PS-K", may also be effective by parenteral or oral routes of administration, especially with. . .

DETD TABLE 1

Acute Neurologic Disorders with Neuronal Damage Thought to be Mediated at Least in Part by Excitatory **Amino Acids***

i. domoic acid poisoning from contaminated mussels
ii. cerebral ischemia, stroke
iii. hypoxia, anoxia, carbon monoxide poisoning
iv. hypoglycemia
v. prolonged epileptic seizures
vi. mechanical trauma. . .

DETD TABLE 2

Chronic Neurodegenerative Diseases with Neuronal Damage Thought or Proposed to be Mediated at Least in Part by Excitatory **Amino Acids**.*

i. Neurolathyrism-BOAA (.beta.-N-oxalylamino-L-**alanine**) in chick peas
ii. Guam Disease-BMAA (.beta.-N-methyl-amino-L-**alanine**) in flour from cycad seeds
iii. Hungtington's disease
iv. ALS (amyotrophic lateral sclerosis)
v. Parkinsonism
vi. Alzheimer's disease
vii. AIDS dementia complex (HIV-associated cognitive/motor complex)
viii.. . .

DETD TABLE 3

Nitric Oxide Synthase Inhibitors:

1. **Arginine** analogs including N-mono-methyl-L-**arginine** (NMA)
2. N-amino-L-**arginine** (NAA)
3. N-nitro-L-**arginine** (NNA)
4. N-nitro-L-**arginine** methyl ester
5. N-iminoethyl-L-ornithine
6. Diphenylene iodonium and analogs See, Steuhr, FASEB J 5:98-103 (1991)
7. Diphenyliodonium, calmodulin inhibitors such as trifluoparizine, calmidazolium. . .

DETD . . . but also to reduce neuronal damage associated with cerebral ischemia, which is mediated by the N-methyl-D-aspartate (NMDA) subtype of excitatory **amino acid** receptor. Bormann et al. reports that certain adamantine derivatives ". . . exhibit NMDA receptor channel-antagonistic and anticonvulsive properties." (2:61-63).. . .

DETD Turski et al. (Nature 349:414, 1991), reports certain experiments investigating the role of excitatory **amino acids** in dopaminergic toxicity caused by intake of a toxin known as MPTP (1-methyl-4-phenyl-1,2,3,6,-tetra hydropyridine). Excitatory **amino acid** antagonists were coadministered with MPP.sup.+ (the active metabolite of MPTP), and certain NMDA antagonists offered temporary protection against MPP.sup.+.

DETD Meldrum, Trends Pharm. Sci. September, 1990, vol. 11, pp. 379-387 reviews reported literature concerning the possibility that excitatory **amino acid** receptor agonists of endogenous or

environmental origin contribute to neuronal degeneration in disease states. After reviewing the several known receptors implicated in excitatory **amino acid** activity (particularly glutamate activity), the authors review (p. 386) suggestions that excitotoxic mechanisms might play a role in the pathogenesis. . .

DETD Choi, Neuron 1:623-634 report that neurotoxicity due to excitatory **amino acids** may be involved in slowly progressive degenerative diseases such as Huntington's disease.

DETD . . . symptoms of the AIDS related complex or acquired immunodeficiency syndrome; the neurotoxicity is mediated (directly or indirectly) by an excitatory **amino acid**, or a structurally similar compound such as quinolinate, which leads to the activation of an NMDA receptor-operated ionic channel; for example, the neurotoxicity is mediated by glutamate, aspartate, homocysteic acid, **cysteine** sulphinic acid, cysteic acid, quinolinate, or N-acetyl aspartyl glutamate.

DETD . . . neuron disease), acquired immunodeficiency (AIDS). Other conditions that may be treated in accordance with the invention include:

neurolathyrism (resulting from .beta.-N-oxalyamino-L-**alanine** found in chick peas); "Guam disease" (resulting from .beta.-N-methyl-amino-L-**alanine** found in flour from cycad seeds); and olivo-pontocerebellar atrophy. The invention also includes therapies for certain mitochondrial abnormalties or inherited. . .

DETD . . . (see below); and that this reduction in damage is due to a block of NMDA receptor-operated channel activation by excitatory **amino acids** (such as glutamate-related compounds) using concentrations of memantine that are readily obtainable in human patients taking the drug (Wesemann et. . . their treatment. In addition to glutamate itself, neuronal injury may result from stimulation of the NMDA receptor by other excitatory **amino acids** or structurally similar compounds; examples of such compounds are aspartate, homocysteic acid, **cysteine** sulphinic acid, cysteic acid, and quinolinate. Neuronal injury may also result from stimulation by excitatory peptides, such as N-acetyl aspartyl. . .

DETD . . . receptor-mediated injury, e.g., that injury resulting from stimulation of the NMDA receptor by glutamate (as shown below) or other excitatory **amino acids** or structurally similar compounds or from stimulation by excitatory peptides, such as N-acetyl aspartyl glutamate.

DETD . . . minimum essential medium (MEM, catalog #1090, Gibco, Grand Island, N.Y.) supplemented with 0.7% (w/v) methylcellulose, 0.3% (w/v) glucose, 2 mM **glutamine**, 1 .mu.g/ml gentamicin, and 5% (v/v) rat serum, as described in Lipton et al., J. Physiol. 385:361, 1987. The cells. . .

DETD . . . culture dishes. The growth medium was Eagle's minimum essential medium supplemented with 0.7% (w/v) methylcellulose, 0.3% (w/v) glucose, 2 mM **glutamine**, 5% (v/v) rat serum, and 1 .mu.g/ml gentamicin. Retinal ganglion cells were identified by the presence of the retrogradely transported. . .

AN 1998:48463 USPATFULL

TI Method of preventing NMDA receptor complex-mediated Neuronal damage

IN Lipton, Stuart A., Newton, MA, United States

PA The Children's Medical Center Corporation, Boston, MA, United States (U.S. corporation)

PI US 5747545 19980505 <--

AI US 1995-407973 19950322 (8)

RLI Continuation of Ser. No. US 1993-25028, filed on 2 Mar 1993, now patented, Pat. No. US 5455279 which is a continuation-in-part of Ser. No. US 1992-949342, filed on 22 Sep 1992, now patented, Pat. No. US 5234956 And Ser. No. US 1992-939824, filed on 3 Sep 1992, now patented, Pat. No. US 5334618 which is a continuation-in-part of Ser. No. US

1991-680201, filed on 4 Apr 1991, now abandoned , said Ser. No. US -949342 which is a continuation of Ser. No. US 1991-688965, filed on 19 Apr 1991, now abandoned
DT Utility
EXNAM Primary Examiner: Lesmes, George F.; Assistant Examiner: Harrison, Robert H.
LREP Fish & Richardson P.C.
CLMN Number of Claims: 1
ECL Exemplary Claim: 1
DRWN 12 Drawing Figure(s); 7 Drawing Page(s)
LN.CNT 1398
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 57 OF 82 USPATFULL
TI Pharmaceutical composition containing R-.**alpha**.-**lipoic acid** or S-.**alpha**.-**lipoic acid** as active ingredient
PI US 5728735 19980317 <--
AB Pharmaceutical compositions and processes for their preparation containing R-.**alpha**.-**lipoic acid** or S-.**alpha**.-**lipoic acid** or pharmaceutically acceptable salts thereof. The pharmaceutical compositions have a cytoprotective activity and are suitable for combatting pain and inflammation.
SUMM The present invention relates to pharmaceutical compositions containing R-.**alpha**.-**lipoic acid** or S-.**alpha**.-**lipoic acid** as an active ingredient. The compositions are useful because they inhibit, for example, acute inflammation as well as inflammatory pain. . .
SUMM .**alpha**.-**lipoic acid** is 1,2-dithiacyclopentane-3-valeric acid. .**alpha**.-**lipoic acid** is widely distributed in plants and animals in the form of the R-enantiomer; it acts as coenzyme in many enzymatic. . . a growth factor for a number of bacteria and protozoa and is used in death-head fungus poisoning. In addition, the .**alpha**.-**lipoic acid** racemate displays anti-inflammatory, antinociceptive (analgesic) and cytoprotective properties.
SUMM It has now surprisingly been found that, in the case of the purely optical isomers of .**alpha**.-**lipoic acid** (R- and S-form, i.e. R-.**alpha**.-**lipoic acid** and S-.**alpha**.-**lipoic acid**), unlike the racemate, the R-enantiomer mainly has an anti-inflammatory activity and the S-enantiomer mainly has an antinociceptive activity, the anti-inflammatory. . .
SUMM The following differences exist in particular in comparison to .**alpha**.-**lipoic acid**, i.e. to the racemate:
SUMM The R-enantiomer acts mainly as an anti-inflammatory and the S-enantiomer mainly as an analgesic, the optical isomers of .**alpha**.-**lipoic acid** being a number of times stronger (for example by at least a factor of 5) than the racemate of -lipoic. . .
SUMM The invention relates to pharmaceutical compositions containing as active ingredient either R-.**alpha**.-**lipoic acid** or S-.**alpha**.-**lipoic acid** (i.e. the optical isomers of .**alpha**.-**lipoic acid**) or a pharmaceutically acceptable salt of these optical isomers of .**alpha**.-**lipoic acid**, the preparation thereof and the use of the optical isomers of .**alpha**.-**lipoic acid** or salts thereof for the preparation of appropriate pharmaceutical compositions. These are particularly suitable for combatting pain and inflammation. A. . .
SUMM The amounts by weight set out herein relate, in each case, to the purely optical isomers of .**alpha**.-**lipoic acid**, i.e. not to the salts. When salts are used, the appropriate amounts must

correspond in each case to the amounts. . .

SUMM The optical isomers of .**alpha.-lipoic acid**, i.e. R-.**alpha.-lipoic acid** and S-.**alpha.-lipoic acid** are preferably used as free acids. In aqueous solutions the salts are preferably used with pharmaceutically acceptable salt formers.

SUMM The preparation of R-.**alpha.-lipoic acid** and S-.**alpha.-lipoic acid** and of salts thereof is effected in known manner or in an analogous manner.

SUMM Salt formers that may be considered for R-.**alpha.-lipoic acid** and S-.**alpha.-lipoic acid** are, for example, conventional bases or cations which are physiologically acceptable in the salt form. Examples include: alkali metals or alkaline earth metals, ammonium hydroxide, basic **amino acids** such as **arginine** and lysine, amines having the formula NR.sub.1 R.sub.2 R.sub.3 in which the radicals R.sub.1, R.sub.2 and R.sub.3 are the same. . . ethylenediamine or hexamethylene tetramine, saturated cyclic amino compounds having 4-6 ring carbon atoms such as piperidine, piperazine, pyrrolidine, morpholine; N-methylglucamine, **creatine**, tromethamine.

SUMM . . . the acid writhing pain test in the mouse and in the Randall-Selitto inflammatory pain test in the rat, the S-enantiomer (S-.**alpha.-lipoic acid**) displays an analgesic activity (peroral application) which is superior by at least a factor of 5 or 6 to that of .**alpha.-lipoic acid** (i.e. the racemate).

SUMM Thus, for example, the above mentioned acid writhing test yielded an analgesically active ED.sub.50 of the S-.**alpha.-lipoic acid** of 10.2 mg/kg per os (ED.sub.50 of the racemate 51.3 mg/kg per os). In the above mentioned Randall-Selitto test, the analgesically effective ED.sub.50 of S-.**alpha.-lipoic acid** is 7.5 mg/kg per os (ED.sub.50 of the racemate 45.9 mg/kg).

SUMM In, for example, carragheen-induced oedema in the rat the R-enantiomer (R-.**alpha.-lipoic acid**) shows an anti-inflammatory activity (peroral application) which is superior by at least a factor of 10 to that of racemic .**alpha.-lipoic acid.**

SUMM The minimum analgesically effective dose of S-.**alpha.-lipoic acid** in the Randall-Selitto pain test is, for example, 1 mg/kg per os.

SUMM The minimum anti-inflammatorily effective dose of R-.**alpha.-lipoic acid** in the carragheen-induced oedema test is, for example, 1 mg/kg per os.

SUMM In addition, R- and S-.**alpha.-lipoic acid** surprisingly possess a growth-inhibiting activity against retroviruses, in particular human immunodeficiency virus HIV (HIV-1, HIV-2) and are, therefore, also suitable. . .

SUMM . . . general between 50 mg to 3 g as a single dose, preferably 100 mg to 1 g of R- or S-.**alpha.-lipoic acid.** The dose per kg of body weight should be between 3.5 and 200 mg, preferably between 7 and 100 mg,. . .

SUMM The daily dose of R- or S-.**alpha.-lipoic acid** in humans should for example be between 70-80 mg per kg weight; the single dose for example 16-20 mg per. . . weight, this dose appropriately being given 4 times daily: the pharmaceutical compositions therefore preferably contain 1-1.5 g of R- or S-.**alpha.-lipoic acid** in a pharmaceutical formulation, a dose of this type preferably being given 4 times each day.

SUMM . . . be used in human medicine alone or in a mixture with other pharmacologically active ingredients. The active ingredients R- or S-.**alpha.-lipoic acid** may also be combined with

any other agent effective against retroviruses, in particular HIV, for example with didesoxyinosin, didesoxycytidine, in. . .

SUMM The dose amounts mentioned refer, in each case, to the free acids R- or S-.**alpha.-lipoic acid**. Should these be used in the form of their salts, the quoted dosages/dosage ranges should be increased in accordance with. . .

SUMM For the combination of R- or S-.**alpha.-lipoic acid** with the component b for example AZT, the two components may in each case be mixed for example in a. . .

SUMM In the case of a combination of R- or S-.**alpha.-lipoic acid** and .alpha.-interferon the two components may for example be present in the following ratio: 50 mg-6 g of R- or S-.**alpha .-lipoic acid** (component a) to 8.times.10.sup.6 enzyme units to 1.times.10.sup.5 enzyme units of .alpha.-interferon, in particular 0.5-3 g of component a to. . .

SUMM In the combination of R- or S-.**alpha.-lipoic acid** and other components in accordance with b), both components may be present as a mixture. In general, the components are,. . .

SUMM . . . the combination to be administered at different times. In such cases it is for example possible to give R- or S-.**alpha.-lipoic acid** as a permanent infusion (dose for example 2-5 g per day) and the other component b to be given at. . . for example 50-800 mg or 1-8.times.10.sup.6 enzyme units, preferably intramuscularly) or also as permanent infusion per day or R- or S-.**alpha.-lipoic acid** may, for example, be given 4 times daily (single dose for example 0.5-2 g) and the other component b at. . .

SUMM For the analgesic activity the general dose range of S-.**alpha .-lipoic acid** that may be considered is, for example:

SUMM For the anti-inflammatory and cytoprotective activity the general dose range of R-.**alpha.-lipoic acid** that may be considered is, for example:

SUMM Apart from its antinociceptive (analgesic) main activity, S-.**alpha.-lipoic acid** also possesses an anti-inflammatory and cytoprotective activity, however to a lesser extent.

SUMM In addition to the main anti-inflammatory and anti-arthritic activity, R-.**alpha.-lipoic acid** also has antinociceptive and cytoprotective activity, albeit to a lesser extent.

SUMM The optical isomers of .**alpha.-lipoic acid** display a good analgesic, anti-inflammatory, anti-arthrotic and cytoprotective activity in, for example, the following investigatory models:

SUMM The optical isomers of .**alpha.-lipoic acid** inhibit for example acute inflammation as well as inflammatory pain and they possess a specific cytoprotective activity.

SUMM . . . are, for example, 0.1 to 600 mg, preferably 15 to 400 mg and in particular 50 to 200 mg of R-.**alpha.-lipoic acid** or S-.**alpha.-lipoic acid**.

SUMM In accordance with the invention the optical isomers of .**alpha .-lipoic acid** (R- or S-form in each case) are given in a daily dose of 10-600 mg, for example of 25 to. . .

SUMM For example the preferred daily dose of both R-.**alpha.-lipoic acid** and S-.**alpha.-lipoic acid** is preferably 80 mg for the parenteral form of application and 200 mg for the oral form. In particular the. . .

SUMM R-.**alpha.-lipoic acid** and S-.**alpha .-lipoic acid** may in particular also be applied in the form of a solution, for example perorally, topically, parenterally (intravenously, intraarticularly, intramuscularly,. . .

SUMM Pharmaceutical compositions containing R-.**alpha.-lipoic acid** or S-.**alpha.-lipoic acid** as active ingredient may for example be formulated in the

form of tablets, capsules, pills or coated tablets, granulates, pellets,. . . for example 0.5 to 20% by weight, preferably 1 to 10% by weight of one of the optical isomers of **.alpha.-lipoic acid** (in each case either the R-form or S-form).

SUMM The dosage unit of the pharmaceutical composition with the optical isomers of **.alpha.-lipoic acid** or a therapeutically useful salt thereof (in each case either the R-form or the S-form) may, for example, contain:

SUMM 10 to 600 mg, preferably 20 to 400 mg, in particular 50 to 200 mg of the optical isomers of **.alpha.-lipoic acid**. The doses may for example be given 1 to 6 times, preferably 1 to 4 times, in particular 1 to. . .

SUMM 10 to 300 mg, preferably 15 to 200 mg, in particular 20 to 100 mg of the optical isomers of **.alpha.-lipoic acid**. The doses may, for example, be given 1 to 6 times, preferably 1 to 4 times, in particular 1 to. . .

SUMM 10 to 500 mg of R-**.alpha.-lipoic acid** or S-**.alpha.-lipoic acid**, preferably 40 to 250 mg, in particular 50 to 200 mg. These doses may for example be administered 1 to. . .

SUMM 0.1 to 300 mg, preferably 0.25 to 150 mg, in particular 0.5 to 80 mg of R-**.alpha.-lipoic acid** or S-**.alpha.-lipoic acid**. These doses may, for example, be administered 1 to 6 times, preferably 1 to 4 times, in particular 1 to. . .

SUMM Should lotions be used, the optical isomers of **.alpha.-lipoic acid** are preferably used in the form of a salt.

SUMM . . . capsules contain 20 to 500 mg, pellets, powders or granulates 20 to 400 mg, suppositories 20 to 300 mg of R-**.alpha.-lipoic acid** or S-**.alpha.-lipoic acid**.

SUMM To combat retroviruses (for example AIDS) the daily dose is for example 4-6 g. Corresponding pharmaceutical compositions therefore preferably contain R-**.alpha.-lipoic acid** or S-**.alpha.-lipoic acid** in the single dose (dose unit) for example in an amount of 600 mg to 1.5 g.

SUMM The above mentioned dosages always relate to the free optical isomers of **.alpha.-lipoic acid**. Should the optical isomers of **.alpha.-lipoic acid** be used in the form of a salt, the dosages/dosage ranges should be correspondingly increased due to the higher molecular. . .

SUMM The acute toxicity of R-**.alpha.-lipoic acid** and S-**.alpha.-lipoic acid** in the mouse (expressed as the LD50 mg/kg; method of LITCHFIELD and WILCOXON, J. Pharmacol. Exp Ther. 95, 99 (1949)),. . .

SUMM In the event of the optical isomers of **.alpha.-lipoic acid** being used in animals, the following indications may be considered in particular: hepatoses, Arthrosis deformans, arthritis and dermatitis.

SUMM The individual optical isomers of **.alpha.-lipoic acid** are suitable for the preparation of pharmaceutical compositions and formulations. The pharmaceutical compositions and/or pharmaceutical compositions contain the optical isomers of **.alpha.-lipoic acid** as active ingredient, optionally in a mixture with other pharmacologically and/or pharmaceutically active ingredients. The preparation of the pharmaceutical compositions. . .

SUMM The pharmaceutical and galenic treatment of the R- or S-**.alpha.-lipoic acid** is carried out using conventional standard methods. For example R- or S-**.alpha.-lipoic acid** and auxiliary or carrier substances may be well mixed by stirring or homogenization (for example using conventional mixing apparatus), working. . .

SUMM Application of the R- or S-.**alpha.-lipoic acid** or of the pharmaceutical compositions may be to the skin or mucous membrane or to the inside of the body,. . .

SUMM If the R- or S-.**alpha.-lipoic acid** are used in the form of their salts, the salt formers may also be used in excess, i.e. in an. . .

SUMM Complex formers that may be considered include those which enclose the R- or S-.**alpha.-lipoic acid** acid in a hollow space. Examples hereof are urea, thiourea, cyclodextrins, amylose. The active molecule substance may optionally be stabilized. . .

SUMM Antioxidants that may for example be used are sodium sulphite, sodium hydrogen sulphite, sodium metabisulphite, **ascorbic acid**, ascorbyl palmitate, -myristate, -stearate, gallic acid, gallic acid alkyl ester, butylhydroxyanisol, nordihydroguaiacic acid, **tocopherols** as well as synergists (substances that bind heavy metals through complex formation, for example lecithin, **ascorbic acid**, phosphoric acid ethylene diamine tetraacetic acid, citrates, tartrates). The addition of the synergists substantially enhances the antioxygenic activity of the. . .

DETD Tablets Containing 50 mg of S- or R-.**alpha.-Lipoic Acid**

DETD 250 g of S-.**alpha.-lipoic acid** are evenly ground with 750 g of microcrystalline cellulose. After sieving the mixture, 250 g of starch (starch 1500/Colorcon), 732.5. . .

DETD Each tablet contains 50 mg of S-.**alpha.-lipoic acid.**

DETD In similar manner it is possible to prepare tablets containing 50 mg of R-.**alpha.-lipoic acid** when the 250 g of lipoic acid is replaced by the same amount of R-.**alpha.-lipoic acid.**

DETD Ampoules Containing 50 mg of S- or R-.**alpha.-Lipoic Acid** as Tromethamine Salt in 2 ml

DETD 250 g of S-.**alpha.-lipoic acid** are dissolved with stirring together with 352.3 g of tromethamine (2-amino-2- (hydroxymethyl)-1,3-propane diol) in a mixture of 9 liters of. . .

DETD One ampoule contains 50 mg of S-.**alpha.-lipoic acid** as tromethamine salt in 2 ml of injection solution.

DETD The same procedure may be used to prepare ampoules with R-.**alpha .-lipoic acid** by using the same amount of R-.**alpha.-lipoic acid** in place of 250 g of S-.**alpha.-lipoic acid.**

CLM What is claimed is:

1. A pharmaceutical composition consisting essentially of a pharmaceutically effective carrier and, as active ingredient, an effective amount of R-.**alpha.-lipoic acid** or a pharmaceutically acceptable salt thereof.

2. A pharmaceutical composition as set forth in claim 1 which is a solution containing R-.**alpha.-lipoic acid** as active ingredient and also a member of the group consisting of stabilizers and solubilizers.

. . . 4. A pharmaceutical dosage unit containing the pharmaceutical composition set forth in claim 1 in an amount such that the R-.**alpha.-lipoic acid** is in each case present in an amount of 0.1 mg to 6 g.

. . . 5. A pharmaceutical dosage unit containing the pharmaceutical composition set forth in claim 1 in an amount such that the R-.**alpha.-lipoic acid** is in each case present in an amount of 0.1 to 600 mg.

6. A tablet containing the pharmaceutical composition set forth in claim

1 in an amount such that the R-.**alpha**.-**lipoic acid** is in each case present in an amount of between 100 mg and 2 g.

. . . solution suitable for parental administration containing the pharmaceutical composition set forth in claim 1 in an amount such that the R-.**alpha**.-**lipoic acid** is in each case present in an amount of 100 mg-12 g.

. . . solution suitable for parental administration containing the pharmaceutical composition set forth in claim 1 in an amount such that the R-.**alpha**.-**lipoic acid** is in each case present in an amount of 200 mg-6 g.

AN 1998:28114 USPATFULL|
TI Pharmaceutical composition containing R-.**alpha**.-**lipoic acid** or S-.**alpha**.-**lipoic acid** as active ingredient|
IN Ulrich, Heinz, Niedernberg, Germany, Federal Republic of
Weischer, Carl-Heinrich, Bonn, Germany, Federal Republic of
Engel, Jurgen, Alzenau, Germany, Federal Republic of
Hettche, Helmut, Dietzenbach, Germany, Federal Republic of
PA Asta Pharma Aktiengesellschaft, Frankfurt, Germany, Federal Republic of (non-U.S. corporation)
PI US 5728735 19980317 <--
AI US 1997-794310 19970203 (8)
RLI Division of Ser. No. US 1992-935656, filed on 26 Aug 1992, now abandoned
which is a continuation of Ser. No. US 1990-610215, filed on 8 Nov 1990,
now abandoned
PRAI DE 1989-3937323 19891109
DT Utility|
EXNAM Primary Examiner: Fay, Zohreh|
LREP Cushman Darby & Cushman IP Group of Pillsbury Madison & Sutro LLP|
CLMN Number of Claims: 10|
ECL Exemplary Claim: 1|
DRWN No Drawings
LN.CNT 739|
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 58 OF 82 USPATFULL
TI Use of R-(+)-.**alpha**.-**lipoic acid**, R-(-)dihydrolipoic acid and metabolites in the form of the free acid or salts or esters or amides for the preparation. . .
PI US 5693664 19971202 <--
AB The invention relates to the use of R-(+)-.**alpha**.-**lipoic acid**, R-(-)-dihydrolipoic acid or their metabolites, salts, esters and amides for the synthesis of drugs for the
treatment of diabetes mellitus. . .
AB The invention furthermore relates to the use of R-(+)-.**alpha**.-**lipoic acid**, R-(-)-dihydrolipoic acid or their metabolites, as well as their salts, esters and amides for the preparation of drugs for the. . .
SUMM R-(+)-.**alpha**.-**lipoic acid** is the physiologically occurring enantiomer of 1,2-dithiocyclopentane-3-valeric
acid. R-(+)-.**alpha**.-**lipoic acid** is a coenzyme of .alpha.-ketoacid dehydrogenases (pyruvate dehydrogenase, .alpha.-ketoglutarate dehydrogenase, etc.) and acts at a key site in the
sugar and energy metabolism of the cell. In its function as an intramolecular redox system, it is oxidized (.**alpha**.-**lipoic acid**) and reduced (dihydrolipoic acid).
SUMM The racemate is used as a 50/50 mixture of R-(+)-.**alpha**.-

lipoic acid and S-(-)-.**alpha**.-**lipoic**
acid for the treatment of diabetic and alcoholic polyneuropathy, as well as for the treatment of Amanita phalloides poisoning and of. . .

SUMM It is well known that the pharmacological properties of the enantiomers of .**alpha**.-**lipoic acid** differ, for example, with respect to their anti-inflammatory and analgesic effect (European patent EP-A 427 247). It is furthermore reported in the literature that R,S-(+,-)-.**alpha**.-**lipoic**
acid has a blood sugar-lowering effect in the case of alloxan-induced diabetes in the animal model. In this connection, it has. . .

SUMM . . . insulin deficiency or a resistance to the action of insulin (decompensated insulin resistance). Subsequently, numerous metabolic disorders particularly of the **carbohydrate** and fat metabolism occur even in the case of still compensated insulin resistance (reduced effect of insulin without clinically manifest. . .

SUMM Diabetics show increased glycosilation and oxidation of **proteins** with corresponding negative consequences for the patients (Z. Makita et at., Science 258, 651-653, 1992).

SUMM The finding that specifically R-(+)-.**alpha**.-**lipoic**
acid is suitable for the treatment of diabetes mellitus and insulin resistance, while the S-(-)-.**alpha**.-**lipoic**
acid practically is not usable for this, is new and unexpected and not inferable by those skilled in the art. Our own investigations have shown that, in animal experiments, the key enzyme, pyruvate dehydrogenase, surprisingly was inhibited by the S-(-)-.**alpha**
.-**lipoic acid**.

SUMM Surprisingly, it has now been found that preferably R-(+)-.**alpha**
.-**lipoic acid** proves to be suitable for the treatment of diabetes mellitus types I and II and its sequelae and late complications. . .

SUMM Trend after two administrations: Lowered by S-(-)-.**alpha**.-
lipoic acid, increased by R-(+)-.**alpha**.-
lipoic acid

SUMM . . . of the Moellegard Company, Denmark, n=10/group) received 0.3 mL
of neutral 0.12 M (corresponding to 50 mg/kg of body weight) R-(+)-.**alpha**.-**lipoic acid** or S-(-)-.**alpha**
.-**lipoic acid** daily, administered in the vein of the tail. A control group received physiological salt solution. After 7 days, the animals. . .

SUMM . . . the enzyme assay (O. H. Lowry et al. J. Biol. Chem. 256, 815-822, 1951) were carried out as described. The **protein** was measured by the method of Lowry (N. Bashan et al., Am. J. Physiol. m262 (Cell Physiol. 31): C682-690, 1992).

SUMM . . . R enantiomer is comparable to that of insulin (200 nM); however, the two effects are not additive. In contrast to R-(+)-.**alpha**.-**lipoic acid**, the S enantiomer decreases the effect of insulin.

SUMM Glucose Assimilation in Muscle Cells in Conjunction with Insulin (200 nM) S-(-)-.**alpha**.-**Lipoic Acid**, (2.5 mM)

SUMM . . . determine hexose assimilation (.sup.3 H-2-desoxyglucose, 10 .mu.M, 10 minutes). Insulin was added at a concentration of 200 nM and the .**alpha**.-**lipoic acid** enantiomers were added at a concentration of 2.5 mM. After the cells were washed and then
lysed with NaOH, the. . .

SUMM The results can be expressed as pmol/min x mg of **protein**. The experiments were carried out by the method described by U.-M. Koivisto et al., J. Biol. Chem. 266, 2615-2621, 1991.

SUMM R-(+)-.**alpha**.-**Lipoic acid** stimulates the translocation of glucose transporters (Glut 1 and GLUT 4) from the cytosol to the plasma membrane; this is equivalent to an activation. S-(-)-.**alpha**.-**Lipoic acid** has no effect or has an inhibiting effect and appears to lower the total content of

glucose transporters in the. . .

SUMM Effect of Enantiomers of **.alpha.-Lipoic Acid** (2.5 mM) on the Translocation of GLUT1 Glucose Transporters in L6-Myotubes

SUMM Effect of Enantiomers of **.alpha.-Lipoic Acid** (2.5 mM) on the Translocation of GLUT4 Glucose Transporters in L6-Myotubes

SUMM . . . 10% polyacrylamide gel for a Western Blot analysis. The glucose transporters were determined with anti-GLUT1 and anti-GLUT4 antibodies using iodine-labeled **protein** A and autoradiographic detection.

SUMM After four hours of incubation, R-(+)-**.alpha.-lipoic acid** increases the cellular content of GLUT1 and GLUT4 glucose transporters. S-(-)-**.alpha.-Lipoic acid** has no effect or lowers the cellular content.

SUMM . . . diabetes animal model (streptozotocin-induced diabetes), it was now surprisingly observed that R-thioct acid corrects numerous pathologically changed parameters (glycosilated hemoglobin, **protein** oxidation), whereas the S enantiomer exhibits a lesser effect to no effect. Surprisingly and additionally, the mortality of the animal. . .

SUMM **Protein**-Carbonyl Formation in the Lens and Liver

SUMM

Experimental Group	nmol Carbonyl/mg **Protein** Lens	Carbonyl/mg **Protein** Liver (% of Control)
Control	0.513 .+-. 0.051 (n = 3)	100. .+-. 8.9 (n = 6)
R-Thioct acid diet	0.429. . .	

SUMM R-(+)-**.alpha.-Lipoic acid** can thus be regarded a highly specific effective drug for the treatment of diabetes mellitus types I and II as. . . well as of disorders in the insulin sensitivity of the tissue (insulin resistance) and of sequelae and late complications. Moreover, R-(+)-**.alpha.-lipoic acid** can be used in the case of diseases with a reduced glucose transporter content or a defective glucose transporter translocation,. . .

SUMM The R-(+)-**.alpha.-lipoic acid,** R-(-)dihydrolipoic acid or their metabolites (such as bisnor- or tetranor-lipoic acid), as well as their salts, esters, amides are synthesized. . .

SUMM The invention also relates to the use of drugs, which contain the optically pure R-(+)-**.alpha.-lipoic acid,** R-(-)-dihydrolipoic acid or their metabolites as well as their salts, esters and amides, for the treatment for the diseases named. . .

DETD Tablets with 100 mg of R-(+)-**.alpha.-Lipoic Acid**

DETD R-(+)-**.alpha.-Lipoic acid** (250 g) is triturated uniformly with 750 g of microcrystalline cellulose. After the mixture is screened, 250 g of starch. . . dispersed silica are admixed and the mixture is pressed into tablets weighing 800.0 mg. One tablet contains 100 mg of R-(+)-**.alpha.-lipoic acid.** If necessary, the tablets can be coated in a conventional manner with a film, which is soluble or permeable to. . .

DETD Ampules with 250 mg of R-(+)-**.alpha.-Lipoic Acid** as Trometamol Salt in 10 mL of Injection Solution

DETD R-(+)-**.alpha.-Lipoic acid** (250 g), together with 352,3 g of trometamol (2-amino-2-(hydroxymethyl)-1,3-propylene glycol) is dissolved with stirring in a mixture of 9 liters. . . filled under aseptic conditions into 10 mL ampules. In 10 mL of

injection solution, 1 ampule contains 250 mg of R-(+)-.**alpha.-lipoic acid** as the trometamol salt.

CLM What is claimed is:
1. A process for the treatment of insulin resistance comprising administering to a patient an effective amount of pure R-(+)-.**alpha.-lipoic acid**, pure R-(-)-dihydrolipoic acid, amides, salts, metabolites or esters thereof.

AN 97:112492 USPATFULL|
TI Use of R-(+)-.**alpha.-lipoic acid**, R-(-)dihydrolipoic acid and metabolites in the form of the free acid or salts or esters or amides for the preparation of drugs for the treatment of diabetes mellitus as well as of its sequelae|
IN Wessel, Klaus, Frankfurt, Germany, Federal Republic of
Borbe, Harald, Mainz, Germany, Federal Republic of
Ulrich, Heinz, Niedernberg, Germany, Federal Republic of
Hettche, Helmut, Dietzenbach, Germany, Federal Republic of
Bisswanger, Hans, Bodelshausen, Germany, Federal Republic of
Packer, Lester, Orinda, CA, United States
Klip, Amira, Toronto, Canada
PA ASTA Medica Aktiengesellschaft, Dresden, Germany, Federal Republic of (non-U.S. corporation)
PI US 5693664 19971202 <--
AI US 1994-360924 19941221 (8)
PRAI DE 1993-4343593 19931221
DT Utility|
EXNAM Primary Examiner: Weddington, Kevin E.|
LREP Cushman Darby & Cushman, IP Group of Pillsbury Madison & Sutro LLP|
CLMN Number of Claims: 2|
ECL Exemplary Claim: 1|
DRWN No Drawings
LN.CNT 448|
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 59 OF 82 USPATFULL
PI US 5691379 19971125 <--
WO 9427592 19941208 <--
SUMM Under physiological conditions, dihydrolipoic acid, the reduced form of **.alpha.-lipoic acid**, is involved in the regulation of the cellular redox state. Oxidation reactions due to certain highly reactive oxygen compounds have. . .
SUMM . . . due to a balanced antioxidative system. This regulation system involves low molecular compounds such as vitamin A (retinol), vitamin C (**ascorbic acid**), vitamin E (.alpha.-**tocopherol**), uric acid and glutathion as well as special enzymes with antioxidative function. If this system is weakened or chronically overloaded,. . .
SUMM Blockage of the coenzyme **.alpha.-lipoic acid** is known to lead to impaired oxidative metabolism.
DETD . . . as riboflavin (vitamin B) and N-formylkynurenine which, being light sensitisers, trigger radiation-related reactions. As a result thereof, discolorations and covalent **protein** cross-linkage occur in the lens during ageing, but intensified in the case of cataractogenesis (pathological clouding due to grey cataract).. . . higher proportion of hydrogen peroxide is also encountered. Furthermore, if a cataract is present there is also continuous oxidation of **cysteine** and methionine in the lens.
DETD Similar radical-mediated **protein** degradations as occur in the lens tissue also occur in the vitreous body of the eye in various metabolic diseases,. . .
DETD All these processes not only change the **protein** structure and thus the fibrous texture of the vitreous body, and thus the light permeability thereof, but can also induce. . .
DETD . . . solution. The mixture is then centrifuged for 30 min. at 15000

g and the aqueous supernatant that contains the water-soluble **protein** proportion is filtered through sterile filters (0.22 um) into brown vials with screw-top lids (20 ml). Before the vials are. . .

DETD 2. Determination of the lens homogenate-**protein** concentration

DETD The Bio-Rad **Protein** Assay is used for quantitatively recording **proteins** in solutions. The assay corresponds to the method described by Bradford (1976) which is based on the shift in the. . . acid, methanol solution of Comassie Brilliant Blue G 250 of 465 nm to 595 nm when this dyestuff binds to **protein** or amino groups. Bovine serum albumin (BSA) is used as standard **protein**.

DETD . . . and the extinction is determined at 595 nm after 15 min. incubation at room temperature. 0.1 ml solvent of the **protein** is used as the test solution in the assay. The colour reagent is subject to ageing, particularly in dilute form.. . . BSA. The photometrically obtained extinction values are converted on the basis of the calibration curve in the form of mg **protein** per ml lens homogenate. The average **protein** content is 110-130 mg per ml lens homogenate.

DETD 3. Riboflavin-catalysed photooxidation of lens **proteins**

DETD . . . the exposure time, high molecular weight aggregates increasingly being formed. This model reaction stimulates a possible photodynamic change in lens **proteins** during the cataractogenesis.

DETD 1. Determination of free **protein** sulfydryl groups

DETD Lens **proteins** have a comparatively high concentration of free SH-groups. If lens **proteins** are exposed to oxidative stress, the SH group decrease can serve as an indication of the extent of the damage. . .

DETD . . . temperature: after 24 hours about 5-10% have already oxidised after 24 hours. If the SH concentration is related to the **protein** content of the lens homogenate, the following absolute values are obtained:

DETD Lens homogenate fresh: 19.60.+-.1.05 umol SH/g **protein**

DETD Lens homogenate (8 weeks at -20.degree. C.): 16.83.+-.0.44 umol SH/g **protein**

DETD . . . batches were gel-filtered through NAP.TM.-25 columns after incubation in the light, whereby the dihydrolipoic acid was separated from the lens **proteins**. The approximately dihydrolipoic acid-free fraction was now used in the Ellman-determination and the sulfhydryl content determined. The NAP.TM.-25 columns separate in the range of 1 to 5 dkal, i.e. **proteins** with a molecular weight of over 5 kdal are eluted with the elution agent. The result, however, is also that. . . this is balanced by a higher sample aliquot in the Ellman batch. Dihydrolipoic acid inhibits SH-group oxidation of the lens **proteins** in dependence of concentration.

DETD The SH-loss of the control resulting from gel filtration is 7.1 .+-.0.4 mM (approx. 15%). Comparing the **protein** contents before and after gel filtration, a loss of 0.52.+-.0.06 mg/ml (approx. 18%) is found. If the elution of a standard **protein** from the NAP.TM.-25 column is checked, a yield of 98.5.+-.3.4% is obtained in the 3.5 ml eluate. This means, however, that the loss of SH or **protein** of the gel filtered lens homogenate is provoked by separated low molecular components.

DETD . . . which are physiologically acceptable in the salt form. Examples hereof are: acceptable alkaline or alkaline earth metals, ammonium hydroxide, basic **amino acids** such as arginin and lysin, amines of the formula NR.sub.1 R.sub.2 R.sub.3 where the radicals R.sub.1, R.sub.2 and R.sub.3 are. . . diamine or hexamethylene tetramine, saturated cyclic amino compounds with 4-6 ring carbon atoms

such as piperidine, piperazine, pyrrolidine, morpholine; N-methylglucamin, **creatine**, trometamol.

DETD Other antioxidants that may for example be used are sodium sulfite, sodium hydrogen sulfite, sodium metabisulfite, **ascorbic acid**, ascorbyl palmirate, -myristate, -stearate, gallic acid, gallic acid alkyl ester, butylhydroxyanisol, nordihydroguaiaretic acid, **tocopherols** and synergists (substances that bind heavy metals by complex formation, for example lecithin, **ascorbic acid**, phosphoric acid, ethylene diaminotetraacetic acid, citrates, tartrates). Addition of the synergists substantially increases the antioxygenic effect of the antioxidants. Conserving. . .

AN 97:109935 USPATFULL

TI Dihydrolipoic acid as an ophthalmological agent to suppress intolerance reactions in the area between implants and living body tissue

IN Ulrich, Heinz, Niedernberg, Germany, Federal Republic of
Elstner, Erich Franz, Grobenzell, Germany, Federal Republic of

PA ASTA Medica Aktiengesellschaft, Dresden, Germany, Federal Republic of (non-U.S. corporation)

PI US 5691379 19971125 <--
WO 9427592 19941208 <--

AI US 1996-557187 19960315 (8)
WO 1994-EP1110 19940411
19960315 PCT 371 date
19960315 PCT 102(e) date

PRAI DE 1993-4317173 19930522

DT Utility

EXNAM Primary Examiner: Spivack, Phyllis G.

LREP Cushman Darby & Cushman, IP Group of Pillsbury Madison & Sutro LLP

CLMN Number of Claims: 8

ECL Exemplary Claim: 1

DRWN 2 Drawing Figure(s); 1 Drawing Page(s)

LN.CNT 689

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 60 OF 82 USPATFULL

PI US 5691203 19971125 <--

DETD . . . peptides having cell adhesive activity (cell adhesive peptides)
have now known from the analysis of the active site of **proteins** having cell adhesive activity such as fibronectin or laminin. The peptide to be employed in the invention may be any. . .

DETD . . . invention are RGDV (Arg-Gly-Asp-Val), RGDS (Arg-Gly-Asp-Ser), RGDN (Arg-Gly-Asp-Asn), DGEA (Asp-Gly-Glu-Ala) and YIGSR (Tyr-Ile-Gly-Ser-Arg). These peptides may be synthesized from respective **amino acids** by conventional methods.

DETD In this specification, **amino acids**, peptides and protective groups are shown by the following abbreviations:

DETD

Ala:	L-**alanine**	Glu:	L-glutamic acid
Arg:	L-**arginine**	Gly:	L-glycine
Asn:	L-asparagine	Ser:	L-serine
Asp:	L-aspartic acid		
		Tyr:	L-tyrosine
Ile:	L-**Isoleucine**	Val:	L-**valine**
Boc:	t-butyloxycarbonyl		
		OBzl:	benzyl
OcHex:	cyclohexyl	Tos:	tosyl

DETD The medium MCDB 131 comprises 2.67 mg/L of L-**Alanine**, 63.20 mg/L of L-**Arginine**.HCl, 2.67 mg/L of L-**Alanine**, 63.20 mg/L of L-**Arginine**.HCl, 15.01 mg/L of L-Asparagine.H.sub.2 O, 13.31 mg/L of L-Aspartic acid, 35.13 mg/L of L-**Cysteine**.HCl-H.sub.2 O, 4.41 mg/L of L-Glutamic acid, 1461.50 mg/L of L-**Glutamine**, 2.25 mg/L of Glycine, 41.93 mg/L of

L-Histidine.HCl.H.sub.2 O, 65.58 mg/L of L-**Isoleucine**, 131.27 mg/L of L-**Leucine**, 181.65 mg/L of L-Lysine.HCl, 14.92 mg/L of L-Methionine, 33.04 mg/L of L-**Phenylalanine**, 11.51 mg/L of L-Proline, 31.53 mg/L of L-Serine, 11.91 mg/L of L-Threonine, 4.08 mg/L of L-Tryptophan, 18.12 mg/L of L-Tyrosine, 117.15 mg/L of L-**Valine**, 0.00733 mg/L of D-Biotin, 0.602 mg/L of Calcium folinate.5H.sub.2 O, 0.002063 mg/L of .**alpha**.-**Lipoic acid**, 6.11 mg/L of Nicotinamide, 11.91 mg/L of D-Pantothenic acid (hemi-Ca salt), 2.056 mg/L of Pyridoxine.HCl, 0.003764 mg/L of Ribmg/L oflavin,. . . mg/L of Vitamine B.sub.12, 0.1351 mg/L of Adenine, 13.96 mg/L of Choline chloride, 1000.00 mg/L of D-Glucose, 7.21 mg/L of i-**Inositol**, 0.0001611 mg/L of Putrescine, 110.04 mg/L of Sodium pyruvate, 0.02422 mg/L of Thymidine, 235.23 mg/L of CaCl.sub.2.2H.sub.2 O, 298.20 mg/L. . .

DETD The medium MCDB 107 comprises 8.909 mg/L of L-**Alanine**, 210.7 mg/L of L-**Arginine**.HCl, 15.07 mg/L of L-Asparagine.H.sub.2 O, 13.31 mg/L of L-Aspartic acid, 8.78 mg/L of L-**Cysteine**.HCl, 14.71 mg/L of L-glutamic acid, 365.3 mg/L of L-**Glutamine**, 22.521 mg/L of Glycine, 20.97 mg/L of L-Histidine.HCl.H.sub.2 O, 3.939 mg/L of L-**Isoleucine**, 13.12 mg/L of L-**Leucine**, 36.54 mg/L of L-Lysine HCl, 4.476 mg/L of L-Methionine, 4.956 mg/L of L-**Phenylalanine**, 34.53 mg/L of L-Proline, 10.51 mg/L of l-Serine, 11.91 mg/L of L-Threonine, 2.042 mg/L of L-Tryptophan, 5.436 mg/L of L-Tyrosine, 11.72 mg/L of L-**Valine**, 0.0073 mg/L of Biotin, 0.0006 mg/L of Folinic acid, 0.0021 mg/L of Lipoic acid, 6.105 mg/L of Nicotinamide, 0.2383 mg/L. . . mg/L of Ribmg/L oflavin, 0.337 mg/L of Thiamine.HCl, 0.136 mg/L of Cyanocobalamine, 25.32 mg/L of Choline ditertrate, 18.02 mg/L of **Inositol**, 4.0437 mg/L of Adenine.SO.sub.4, 0.0028 mg/L of Linoleic acid, 0.00016 mg/L of Putrescine.2HCl, 0.0727 mg/L of Thymidine, 6640.08 mg/L of. . .

DETD Serum albumin is one kind of **proteins** present in serum, and it has been reported that the serum albumin in serum-free culture enhances the growth of animal. . .

DETD . . . be maintained. By contrast, addition of more than 1000 .mu.g/ml of the albumin is not desirable since removal of, a **protein**, i.e. a serum albumins, requires troublesome procedures in isolating the substances secreted by the cells after the culture.

DETD . . . hydrocortisone, insulin and BBE, in addition to serum albumin and transferrin, are preferably added to the basal medium. When desired, .alpha.-**tocopherol**, cholesterol, and the like may be further added to the medium.

DETD In the same manner as with the RGDV peptide, DGEA and YIGSR peptides were synthesized from their respective component **amino acids**.

DETD . . . part of the polymer [I] was hydrolyzed in 6N hydrochloric acid at 166.degree. C. for 30 minutes and submitted to **amino acid** analysis to confirm that 17.9 .mu.g of RGDV peptide had been introduced into 1 mg of the polymer. The mean. . .

DETD . . . cell adhesive activity was obtained according to the same way as polymer [I] except using 0.1 g of RGDV peptide. **Amino acid** analysis showed that in 1 mg of the polymer [II], 2.1 .mu.g of RGDV peptide was introduced.

DETD . . . according to the same way as polymer [I] except that RGDV peptide was replaced by 0.1 g of DGEA peptide. **Amino acid** analysis showed that in 1 mg of the polymer [III], 3.0 .mu.g of DGEA peptide was introduced. The mean molecular. . .

DETD . . . according to the same way as polymer [I] except that RGDV peptide was replaced by 0.1 g of YIGSR peptide. **Amino acid** analysis showed that in 1 mg of the polymer [IV], 2.4 .mu.g of YIGSR peptide was introduced.

DETD

SEQUENCE LISTING
(1) GENERAL INFORMATION:
(iii) NUMBER OF SEQUENCES: 5
(2) INFORMATION FOR SEQ ID NO:1:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 **amino acids**
(B) TYPE: **amino acid**
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:
ArgGlyAspVal
(2) INFORMATION FOR SEQ ID NO:2:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 **amino acids**
(B) TYPE: **amino acid**
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:
ArgGlyAspSer
1
(2) INFORMATION FOR SEQ ID NO:3:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 **amino acids**
(B) TYPE: **amino acid**
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:
ArgGlyAspAsn
1
(2) INFORMATION FOR SEQ ID NO:4:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 **amino acids**
(B) TYPE: **amino acid**
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:
AspGlyGluAla
1
(2) INFORMATION FOR SEQ ID NO:5:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 **amino acids**
(B) TYPE: **amino acid**
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:
TyrIleGlySerArg
15

CLM What is claimed is:
. . . medium selected from the group consisting of MCDB 131 and MCDB 107, wherein medium MCDB 131 comprises 2.67 mg/L of L-**Alanine**, 63.20 mg/L of L-**Arginine**.HCl, 2.67 mg/L of L-**Alanine** , 63.20 mg/L of L-**Arginine** .HCl, 15.01 mg/L of L-Asparagine H.sub.2 O, 13.31 mg/L of L-Aspartic acid, 35.13 mg/L of L-**Cysteine**.HCl-H.sub.2 O, 4.41 mg/L of L-Glutamic acid, 1461.50 mg/L of L-**Glutamine**, 2.25 mg/L of Glycine, 41.93 mg/L of L-Histidine.HCl.H.sub.2 O, 65.58 mg/L of L-**Isoleucine**, 131.27 mg/L of L-**Leucine**, 181.65 mg/L of L-Lysine.HCl, 14.92 mg/L of L-Methionine, 33.04 mg/L of L-**Phenylalanine**, 11.51 mg/L of L-Proline, 31.53 mg/L of L-Serine, 11.91 mg/L of L-Threonine, 4.08 mg/L

of L-Tryptophan, 18.12 mg/L of L-Tyrosine, 117.15 mg/L of L-**Valine**, 0.00733 mg/L of D-Biotin, 0.602 mg/L of Calcium folinate.5H.sub.2 O, 0.002063 mg/L of .**alpha**.-**Lipoic acid**, 6.11 mg/L of Nicotinamide, 11.91 mg/L of D-Pantothenic acid (hemi-Ca salt), 2.056 mg/L of Pyridoxine.HCl, 0.003764 mg/L of Ribmg/L oflavin,. . . mg/L of Vitamine B.sub.12, 0.1351 mg/L of Adenine, 13.96 mg/L of Choline chloride, 1000.00 mg/L of D-Glucose, 7.21 mg/L of i-**Inositol**, 0.0001611 mg/L of Putrescine, 110.04 mg/L of Sodium pyruvate, 0.02422 mg/L of Thymidine, 235.23 mg/L of CaCl.sub.2.2H.sub.2 O, 298.20 mg/L. . . ZnSO.sub.4.7H.sub.2 O, 1176.0 mg/L of NaHCO.sub.3, 12.42 mg/L of Phenol red (Sodium salt); and MCDB 107 comprises 8.909 mg/L of L-**Alanine**, 210.7 mg/L of L-**Arginine**.HCl, 15.07 mg/L of L-Asparagine.H.sub.2 O, 13.31 mg/L of L-Aspartic acid, 8.78 mg/L of L-**Cysteine**.HCl, 14.71 mg/L of L-Glutamic acid, 365.3 mg/L of L-**Glutamine**, 22.521 mg/L of Glycine, 20.97 mg/L of L-Histidine.HCl.H.sub.2 O, 3.939 mg/L of L-**Isoleucine**, 13.12 mg/L of L-**Leucine**, 36.54 mg/L of L-Lysine HCl, 4.476 mg/L of L-Methionine, 4.956 mg/L of L-**Phenylalanine**, 34.53 mg/L of L-Proline, 10.51 mg/L of l-Serine, 11.91 mg/L of L-Threonine, 2.042 mg/L of L-Tryptophan, 5.436 mg/L of L-Tyrosine, 11.72 mg/L of L-**Valine**, 0.0073 mg/L of Biotin, 0.0006 mg/L of Folinic acid, 0.0021 mg/L of Lipoic acid, 6.105 mg/L of Nicotinamide, 0.2383 mg/L. . . mg/L of Ribmg/L oflavin, 0.337 mg/L of Thiamine.HCl, 0.136 mg/L of Cyanocobalamine, 25.32 mg/L of Choline ditertrate, 18.02 mg/L of **Inositol**, 4.0437 mg/L of Adenine.SO.sub.4, 0.0028 mg/L of Linoleic acid, 0.00016 mg/L of Putrescine.2HCl, 0.0727 mg/L of Thymidine, 6640.08 mg/L of. . .

AN 97:109766 USPATFULL|
TI Method for serum-free culture of human vascular endothelial cells|
IN Katsuen, Susumu, Osaka, Japan
Ohshima, Kunihiro, Osaka, Japan
Yamamoto, Ryohei, Takatsuki, Japan
Nishino, Toyokazu, Ibaraki, Japan
PA Kurashiki Boseki Kabushiki Kaisha, Kurashiki, Japan (non-U.S. corporation)
PI US 5691203 19971125 <--
AI US 1993-128225 19930929 (8)
PRAI JP 1993-141984 19930614
DT Utility|
EXNAM Primary Examiner: Naff, David M.|
LREP Foley & Lardner|
CLMN Number of Claims: 5|
ECL Exemplary Claim: 1|
DRWN 1 Drawing Figure(s); 1 Drawing Page(s)|
LN.CNT 789|
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 61 OF 82 USPATFULL
PI US 5661023 19970826 <--
SUMM . . . a high capital investment for scale-up. The method also only produces about 10 doses of Salk-type immunogen having a total **protein** content of about 100 .mu.g/mL or 8-10 .mu.g/mL by p24 based ELISA per 1 liter of cell culture suspension. Thus,. . .
SUMM Numerous references disclose methods for isolating individual viral **proteins**. WO9113906 discloses fractionating the non-fusion recombinant HIV viral **protein** gp 120 by ion exchange chromatography and purifying the fraction exhibiting CD4 specific binding affinity by hydrophobic interaction and size-exclusion chromatography. U.S. Pat. No. 4,531,311 discloses separating recombinant HIV reverse transcriptase **protein** from contaminating cellular **proteins** by using a cation-exchange resin. JP61051571 discloses purifying a swine herpes virus antigan by its adsorbtion on an ionic

exchange. . . (1990) and C. M. Nalin, et al., J.Cell Biochem. Suppl. 14D, 108 (1990) disclose the purification of recombinant HIV Rev **protein** by ion exchange and gel filtration chromatography. J. Rittenhouse, et al., Biochem. Biophys. Res. Commun. 171 (1) 60(1990) disclose purifying. . . column chromatography. J. E. Newman, et al., Abstr. Annu.Meet.Am. Soc. Microbiol. 86 Meet., 329 T-43 (1986) disclose isolating HTLV-3 structural **proteins** by passage over a lentil lectin affinity resin, followed by further purification using HPLC ion exchange chromatography and gel filtration. . .

DETD "Viral particles" include complete virions (viruses), as well as related viral particles, but not single viral **proteins**. The source of the viral particles for cell culturing may be from a virally infected patient or viruses propagated in. . . passage on susceptible cell systems. Examples of viral particles include capsids, core particles, virions depleted of one or more envelope **proteins**, virion envelopes without the nuclear capsid core, virion envelope fragments and defective or incomplete virions. A preferred viral particles are. . .

DETD . . . their genomic material, including human immunodeficiency viruses (HIV), such as HIV-1 and HIV-2, HIV depleted of gp 120 and/or 160 **proteins**, HTLV-1, HTLV-2, AKR virus AKR-L#1, Moloney leukemia virus, and BLV. Preferred retroviral particles include HIV, HIV depleted of gp 120 and/or 160 **proteins**, HTLV-1 and 25 HTLV-2, and more preferred is HIV-1.

DETD . . . supports the growth and survival of a mammalian cell system. The growth medium may or may not contain serum or **proteins**.

DETD . . . retroviral particles, more preferably the retroviral particles are HTLV-1, HTLV-2, HIV-1, HIV-2 or HIV depleted of gp 120 and/or 160 **proteins**, and further preferably said retroviral particles are HIV-1;

DETD wherein said retroviral particles are HTLV-1, HTLV-2, HIV-1, HIV-2 or HIV depleted of gp 120 and/or 160 **proteins**, more preferably wherein said retroviral particles are HIV depleted of gp 120 or gp 160 **proteins;**

DETD . . . BPL. This step serves to chemically reduce infectivity of the virus by alkylating the various structural components such as lipids, **proteins**, nucleic acid, etc. BPL inactivation is not considered to be a definitive inactivation step, and as such the BPL treated. . .

DETD . . . column .is the most critical and efficient purification step in the process achieving about a 40 fold purification to total **protein** ratio. SDS-PAGE analysis shows the bulk of the medium components are in the column flow through, more contaminants are removed by the wash the NaCl wash, and for HIV particles a recognizable **protein** pattern compared to a HIV-1 reference standard is determinable from the elution fraction.

DETD

Component	Conc.
Transferrin, Human (HOLO) HI (Miles)	0.010 g/L
Insulin, Human recombinant, Zn	0.010 g/L
Albumin, Human Serum (25%)	5.0 mL/L
Cholesterol	0.00045 g/L
DL-Alpha-**Tocopherol** Acetate	0.0002 g/L
Cod Liver Oil	0.001 g/L
Linoleic Acid	0.000021 g/L
DL- **Alpha-Lipoic Acid**	0.0000515 g/L
Tween 80	0.0025 mL/L

Ethanolamine F.B. 0.000611 g/L
Pluronic F-68, NF Grade 1.000 g/L

DETD to RPMI 1640 containing 4 g/L of glucose and 0.3 g/L of L-**glutamine**. The reduction in the **protein** content of the serum-free medium helps in the efficiency of downstream processing.

DETD . . . chemically reduce the infectivity of the viral particles by alkylating the various structural constituents of the virus such as lipids, **proteins**, nucleic acids, etc. We point out that this BPL inactivation step is not a definitive virus inactivation, and the material. . .

DETD . . . used in this step serves to retain HIV particles while removing greater than 90% of human serum albumin and other **proteins** from the growth medium, and other relatively small molecules in the permeate.

CLM What is claimed:

. . . of claim 1 wherein said retroviral particles are HTLV-1, HTLV-2, HIV-1, HIV-2 or HIV depleted of gp 120 and/or 160 **proteins**.

4. The process of claim 1 wherein said retroviral particles are HIV depleted of gp 120 or gp 160 **proteins**.

AN 97:76008 USPATFULL|

TI Production and purification of retroviral particles using tentacle anion exchange|

IN Hrinda, Michael E., Gwynedd Valley, PA, United States
Prior, Christopher P., Wayne, PA, United States
Mitschelen, Jonathan J., Perkiomenville, PA, United States
Irish, Thomas W., Pottstown, PA, United States
Weber, David M., Phoenixville, PA, United States
Gore, Richard S., Southampton, PA, United States
Harter, James J., Media, PA, United States
Bay, Pierre M., Philadelphia, PA, United States
Tarr, George C., Norristown, PA, United States

PA The Immune Response Corporation, Carlsbad, CA, United States (U.S. corporation)

PI US 5661023 19970826 <--

AI US 1996-613920 19960311 (8)

RLI Continuation of Ser. No. US 1994-215833, filed on 22 Mar 1994, now abandoned

DT Utility|

EXNAM Primary Examiner: Lankford, Blaine|

LREP Campbell & Flores LLP|

CLMN Number of Claims: 10|

ECL Exemplary Claim: 1|

DRWN 6 Drawing Figure(s); 6 Drawing Page(s)|

LN.CNT 746|

L7 ANSWER 62 OF 82 USPATFULL

PI US 5578414 19961126 <--

DETD . . . other hydrophilic colloids can also be used. Examples thereof include gelatin derivatives; graft polymers of gelatin and other high polymers; **proteins** such as albumin and casein; cellulose derivatives such as hydroxyethyl cellulose, carboxymethyl cellulose, and cellulose sulfate; sodium alginate; sugar derivatives. . .

DETD The preferred compound represented by formula (E) is an **ascorbic acid** or an erythorbic acid (stereoisomer). The addition amount of the compound represented by formula (E) is from 0.03 to 0.12. . .

DETD . . . usually used as a buffer should not be present in the developing solution because it forms a complex with the **ascorbic acid** derivative compound represented by formula (E).

DETD . . . (e.g., malic acid, tartaric acid, citric acid, succinic acid,

oxalic acid, maleic acid, glycolic acid, benzoic acid, salicylic acid, Tiron, **ascorbic acid**, glutaric acid, adipic acid), **amino acids** (e.g., aspartic acid, glycine, **cysteine**), aminopolycarboxylic acids (e.g., ethylenediaminetetraacetic acid, diethylenetriaminepentaacetic acid, 1,3-propanediaminetetraacetic acid, nitrilotriacetic acid) and saccharides.

DETD TABLE 1

	per m.sup.2	
Lower Protective Layer		
Gelatin	0.5	g
1,5-Dihydroxy-2-benzaldoxime	25	mg
.alpha.-Lipoic Acid	5	mg
Polyethyl Acrylate Latex	160	mg
Upper Protective Layer		
Gelatin	0.3	g
Silica Matting Agent (average size: 2.5 .mu.m)	30	mg
Silicone Oil	30. . .	

AN 96:108786 USPATFULL
TI Silver halide photographic material and method for processing the same
IN Yamamoto, Seiichi, Kanagawa, Japan
Yoshida, Tetsuo, Kanagawa, Japan
Hioki, Takanori, Kanagawa, Japan
PA Fuji Photo Film Co., Ltd., Kanagawa, Japan (non-U.S. corporation)
PI US 5578414 19961126 <--
AI US 1995-423708 19950418 (8)
PRAI JP 1994-103272 19940419
DT Utility
EXNAM Primary Examiner: Chea, Thorl
LREP Sughrue, Mion, Zinn, Macpeak & Seas
CLMN Number of Claims: 6
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 2349
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 63 OF 82 USPATFULL
TI Combination medications containing **alpha-lipoic acid** and related
PI US 5569670 19961029 <--
AB A pharmaceutical composition containing **alpha-lipoic acid**, dihydrolipoic acid, metabolites of **alpha-lipoic acid** (inter alia bisnortetralipoic acid and tetranorlipoic acid), optical isomers R- and S- forms of **alpha-lipoic acid** in oxidized and reduced form together with a vitamin, especially vitamins A, B1, B2, B6, B12, C and E and. . .
SUMM The present invention relates to a synergistic combination of medications containing, as active ingredient, **alpha-lipoic acid**, dihydrolipoic acid, their metabolites as well as the oxidized and reduced enantionmers of **alpha-lipoic acid** such as R-**alpha-lipoic acid** or S-**alpha-lipoic acid** as well as metabolites of **alpha-lipoic acid** together with vitamins, especially vitamins A, B1-6, B12, C and E.
SUMM **Alpha-lipoic acid** is 1,2-dithia-cyclopentane-3-valeric acid.
SUMM **Alpha-lipoic acid** is distributed widely in plants and animals in the form of the R-enantiomer; it acts as a coenzyme in many. . . enzymatic reactions, constitutes a growth factor for certain bacteria and protozoa and is used to treat death-head

mushroom poisoning. The **alpha-lipoic acid** racemate also has anti-inflammatory, antinociceptive (analgesic) and cytoprotective, neuroprotective, anti-allergic and antitumor properties.

SUMM The separated optical isomers of **alpha-lipoic acid** (R- and S-form, i.e. R-**alpha-lipoic acid** and S-**alpha-lipoic acid**), have different properties from each other and from the racemate. The R-enantionmer has a predominantly anti-inflammatory effect, and the S-enantiomer. . .

SUMM . . . system. Its main function is to protect lipids from peroxidation. Japanese published patent 3-193778 describes esters of lipoic acid with **tocopherols**. These **tocopherol** esters of lipoic acid are used to treat UV-erythemas.

DETD These and other objects are provided in medications which contain, as active ingredient, a member of the group consisting of **alpha-lipoic acid**, dihydrolipoic acid, their oxidized or reduced R- or S-isomers, and metabolites of **alpha-lipoic acid** (inter alia, 6,8-bisnorlipoic acid and tetranorlipoic acid), referred to hereinafter as "**alpha-lipoic acid** or related compound," and at least one vitamin or a pharmaceutically acceptable salt thereof. In a preferred form of the. . .

DETD The **tocopherols** (vitamin E) used in the preparation according to this invention can be alpha-**tocopherol**, .beta.-**tocopherol**, gamma-**tocopherol** or delta-**tocopherol**. These can be obtained from natural oils (d-form) as well as from synthetic material (dl-form). It is also possible to use **tocopherol** acetate as well as other esters of physiologically acceptable acids.

DETD . . . surprisingly been found that, in the combination of active substances, such as vitamin E, with the pure optical isomers of **alpha-lipoic acid** (R- and S-form, i.e. R-**alpha-lipoic acid** and S-**alpha-lipoic acid**), in contrast to the racemate of **alpha-lipoic acid** alone, the R-enantiomer has an anti-inflammatory and antidiabetic action, i.e. it reduces blood sugar, and the S-enantiomer has an antinociceptive. . . anti-inflammatory effect of the R-enantiomer in combination with vitamin E is surprisingly also stronger than that of the racemate of **alpha-lipoic acid**. The antinociceptive (analgesic) effect of the S-enantiomer in combination with vitamin E is for example stronger than that of the racemate of **alpha-lipoic acid**. The enantiomers in combination with vitamins A, B1, B2, B6, B12, C and E are therefore very much more specific and stronger acting active substances compared to the racemate of **alpha-lipoic acid**.

DETD There are in particular the following differences compared to **alpha-lipoic acid** (racemate) in combination with vitamins A, B1, B2, B6, B12, C and E, such as the vitamins: in aqueous solutions the salts of the active compounds are preferably used with pharmaceutically acceptable salt formers. This means that the **alpha-lipoic acid** is not employed as the free acid in the pharmaceutical formulation, but it is employed as a salt with a. . .

DETD The preparation of **alpha-lipoic acid**, dihydrolipoic acid or of the oxidized or reduced R-**alpha-lipoic acid** and of S-**alpha-lipoic acid** or the metabolites of **alpha-lipoic acid** as well as their salts in combination with the vitamins listed is carried out in known manner, or by analogy. . .

DETD Salt formers for **alpha-lipoic acid**, diydrolipoic acid, their oxidized or reduced R- or S-isomers, and metabolites of **alpha-lipoic acid** (6,8-bisnorlipoic acid and tetranorlipoic acid) can for example be

conventional bases or cations which are physiologically acceptable in the salt form. Examples include: alkaline or alkaline earth metals, ammonium hydroxide, basic **amino acids** such as **arginine** and lysine, amines of formula NR.sub.1 R.sub.2 R.sub.3 where the radicals R.sub.1, R.sub.2 and R.sub.3 are the same or different. . . diamine or hexamethylene tetramine, saturated cyclic amino compounds with 4-6 ring carbon atoms such as piperidine, piperazine, pyrrolidine, morpholine; N-methylglucamine, **creatine** and tromethamine.

DETD . . . acetic acid writhing pain test in the mouse and the Randall Selitto inflammation pain test in the rat the S-enantiomer (S-**alpha-lipoic acid**) in the combination with vitamin E for example shows an analgesic effect which is superior to that of **alpha-lipoic acid** alone (i.e. the racemate) or of vitamin E alone (peroral administration).

DETD In carragheen-edema in the rat, the R-enantiomer (R-**alpha-lipoic acid**) in combination with vitamin E for example shows an anti-inflammatory effect which is superior to that of **alpha-lipoic acid** (alone) or to vitamin E alone (peroral administration).

DETD . . . a cytoprotective effect is for example apparent in animal experiments both for the oxidized or reduced R- and S-form of **alpha-lipoic acid** in combination with vitamin E starting from a dose as low as 20 mg/kg R- and S-isomer of **alpha-lipoic acid** in combination with 50 mg/kg vitamin E per os.

DETD In the alloxan diabetes model or the streptocytozine diabetes model the R-enantiomer (R-**alpha-lipoic acid**) in combination with vitamin E for example displays for example an antidiabetic, i.e. blood sugar-reducing effect, which is superior to that of **alpha-lipoic acid** (alone) or to vitamin E alone (peroral administration).

DETD In the rat, the R-enantiomer (R-**alpha-lipoic acid**) in combination with vitamin E displays for example a liver enzyme-regulating effect which is superior to that of **alpha-lipoic acid** (alone) or to vitamin E alone (peroral administration).

DETD . . . thus a detoxifying effect, is for example present both for the oxidized or reduced racemates or R- and S-form of **alpha-lipoic acid** in combination with vitamin E from as low a dose as 30 mg/kg R- or S-isomer of **alpha-lipoic acid** in combination with 50 mg/kg vitamin E per os.

DETD . . . an immune stimulating effect occurs for example in animal experiments both for the oxidized or reduced R- and S-form of **alpha-lipoic acid** in combination with vitamin E from as low as dose as 35 mg/kg R- or S-isomer of **alpha-lipoic acid** in combination with 50 mg/kg vitamin E per os.

DETD In addition, R- and S-**alpha-lipoic acid** in combination with vitamin E have a growth inhibiting effect against retroviruses, in particular the human immune deficiency virus HIV. .

DETD The combinations of **alpha-lipoic acid**, dihydrolipoic acid, their metabolites as well as the oxidized and reduced enantionmers of **alpha-lipoic acid** such as R-**alpha-lipoic acid** or S-**alpha-lipoic acid** as well as metabolites of **alpha-lipoic acid** with the vitamins A, B 1-6, B12, C and E display a good analgesic, anti-inflammatory, anti-arthrotic and cytoprotective effect in. . .

DETD The acute toxicity of R-**alpha-lipoic acid** and S-**alpha-lipoic acid** in the mouse (expressed as the LD.sub.50 mg/kg; LITCHFIELD and WILCOXON method, J. Pharmacol. Exp. Ther. 95, 99 (1949)). . .

DETD

TOXICITY

alpha-lipoic acid (racemate)

LD.sub.50 p.o.mg/kg	Species
502	mouse, male
460	mouse, female
1190	rat, male
1210	rat, female

Vitamin B1

LD.sub.50 p.o.mg/kg	i.v.mg/kg	Species
8200-13300		
. . . Species		
8021	1058	mouse
>5000	1000	rat

Vitamin E

LD.sub.50

p.o.mg/kg	i.v.mg/kg	Species
>50,000	>2100	mouse
>5000	>1500	rat

Examples: TOXICOLOGY OF THE COMBINATIONS

alpha-lipoic acid (racemate) with 30 mg/kg vitamin E

LD.sub.50

p.o.	Species
>1200 mg	mouse

for **alpha-lipoic acid**

Vitamin E (30 mg/kg p.o.) with R-enantiomer of **alpha-lipoic acid**

LD.sub.50

p.o.	Species
>1200 mg for the R-enantiomer of **alpha-lipoic acid**	mouse

Vitamin E (30 mg/kg p.o.) with S-enantiomer of **alpha-lipoic acid**

LD.sub.50

p.o.	Species
>1200 mg for the S-enantiomer of **alpha-lipoic acid**	mouse

DETD . . . generally contain between 1 mg and 3 g as a single dose, preferably 2 mg to 1.2 g R- or S-**alpha-lipoic acid** for example in combination with 1 to 450 mg vitamin E. The active substance levels/kg body weight achieved should be between 1.5 and 200 mg for R- and S-**alpha-lipoic acid**, preferably between 4 and 100 mg, in particular between 8 and 70 mg/kg for the R- or S-form of **alpha-lipoic acid** and for example for the vitamin E preferably between 0.01 and 20 mg/kg BW, particularly between 0.1 and 8 mg/kg. . .

DETD . . . oral doses to treat heavy metal intoxication in humans

Alpha-lipoic acid Daily Dose of **Alpha-lipoic acid** or related compound	Vitamin E or related compound Daily Dose of Vitamin	Single Dose of (a) Alpha-lipoic acid or related compound/ Administration (b) Vitmain	Frequency of (per day)
oxide/reduc. race-			

Vitamin A
300. . . B1
300 mg-1.2 g
5-50 mg (a) 100 mg-400 mg
1-4
mate or R- or S- (b) 1 mg-12 mg
isomer of **alpha-**
lipoic acid
oxide/reduc. race-
Vitamin B6
300 mg-1.2 g
5-50 mg (a) 100 mg-400 mg
1-4
mate or R- or S- (b) 1 mg-12 mg
isomer of **alpha-**
lipoic acid
oxide/reduc. race-
Vitamin B12
300 mg-1.2 g
5-50 micrograms
(b) 1 microgram-
1-4
mate or R- or S- 12 micrograms
isomer of alpha-. . . C
300 mg-1.2 g
200-1,000 mg
(a) 100 mg-400 mg
1-4
mate or R- or S- (b) 50 mg-250 mg
isomer of **alpha-**
lipoic acid
oxide/reduc. race-
Vitamnin E
250 mg-1.2 g
100-800 mg
(a) 60 mg-400 mg
1-4
mate or R- or S- (b) 25 mg-200 mg
isomer of **alpha-**
lipoic acid

DETD The single dose of active substance of the **alpha lipoic acid** or related compound in the combination for example with vitamin E can for example be:

DETD The daily dose of R- or S-**alpha-lipoic acid** in the combination for example with vitamin E in man may for example be 2-40 mg per kg weight; the. . .

DETD The daily dose may for example be between 100-600 mg: the medications therefore preferably contain 100-600 mg of R- or S-**alpha-lipoic acid** in a pharmaceutical formulation, a dose of this kind preferably being given up to 4 times per day.

DETD . . . example possible to recommend 1 to 4 tablets, 3 times daily, with a content of 10 mg to 2 g **alpha lipoic acid** or related compound or for example in intravenous injection 1 to 4 times daily one ampoule/infusion vial of 1 to 100 ml content with 200 mg to 6 g **alpha-lipoic acid** or related compound in combination with 0.001-2 g of vitamin.

DETD In oral administration, the minimum daily dose of the **alpha lipoic acid** or related compound in combination with the vitamin is for example 100 mg; the maximum daily dose in oral administration. . .

DETD The single dose of the vitamin in combination with the R- or S-isomer of **alpha-lipoic acid** can for example be, in the case of vitamin E:

DETD The daily oral dose of vitamin E in combination with the oxidized or reduced racemate or R- or S-isomer of **alpha-lipoic acid** in man can for example be 0.1-12 mg/kg body weight; the single dose of vitamin E in the combination for. . .

DETD . . . be used in human medicine alone or in a mixture with other pharmacologically active substances. The active substances R- or S-**alpha-lipoic acid** can also be combined with any other agent active against retroviruses, in particular HIV, for example didesoxyinosine, didesoxycytidine, however in. . .

DETD The dose amounts cited of the **alpha-lipoic acid** or related compound always relate to the free acids of **alpha-lipoic acid**, dihydrolipoic acid or of oxidized or reduced R- or S-**alpha-lipoic acid**. Should these be used in the form of their salts, the stated dosages/dosage ranges should be correspondingly increased to the.

. .

DETD It is for example possible for the combination of vitamin E with R- or S-**alpha-lipoic acid** with the component b for example AZT to mix the two components in each case for example in a ratio. . . to 3 up to 3 to 1 parts. In the case of a combination of vitamin E with R- or S-**alpha-lipoic acid** and alpha-interferon the three components may be present for example in the following ratios: 15 mg-50 mg-6 g R- or S-**alpha-lipoic acid** (component (a)) to 8.times.10.sup.6 enzyme units to 1.times.10.sup.5 enzyme units alpha-interferon, in particular 0.5-3 g component (a) to 1-4.times.10.sup.6 enzyme units alpha-interferon. In the combination of for example vitamin E with R- or S-**alpha-lipoic acid** and other components according to b) both components may be present as a mixture. In general the components are however. . .

DETD . . . to be administered simultaneously. In such cases it is, for example, possible to give vitamin E intramuscularly and R- or S-**alpha-lipoic acid** as long-term infusion (dose for example 2-5 g per day) and the third component (b) simultaneously (dose for example 50-800 mg or 1-8.times.10.sup.6 enzyme units, preferably intramuscular) or also as long-term infusion per day or R- or S-**alpha-lipoic acid** can for example be given 4 times daily (single dose for example 0.5-2 g) and the other component (b) simultaneously. . .

DETD . . . HIV viruses, appropriate medications should also contain such an amount of for example vitamin E in combination with R- or S-**alpha-lipoic acid** or these should be given in such amounts that single or multiple administration results in an active level of vitamin. . .

DETD The general dose range for the combinations with the above mentioned vitamins with R- or S-**alpha-lipoic acid** for analgesic effect is for example: 0.5-20 mg/kg body weight oral vitamin E in combination with 1-100 mg/kg body weight R- or S-isomer of **alpha-lipoic acid**.

DETD The general dose range of combinations with the above mentioned vitamins with R-**alpha-lipoic acid** for anti-inflammatory and cytoprotective effect is for example: 0.5-15 mg/kg body weight oral vitamin E in combination with 1-100 mg/kg body weight R- or S-isomer of **alpha-lipoic acid**

DETD The general dose range of combinations with the above mentioned vitamins with R-**alpha-lipoic acid** for the detoxifying, heavy metal antidote effect one can use for example: 0.5-25 mg/kg body weight oral vitamin E in combination with 1-100 mg/kg body weight R- or S-isomer of **alpha-lipoic acid**

DETD The general dose range of combinations with the above mentioned vitamins with R-**alpha-lipoic acid** for the anti-allergic and immune-stimulating effect one can use for example: 0.5-20 mg/kg body weight oral vitamin E in combination with 1-100 mg/kg body weight R- or S-isomer of **alpha-lipoic acid**

DETD The general dose range of combinations with the above mentioned vitamins with R-**alpha-lipoic acid** for the antitumor effect one can use for example: 0.5-25 mg/kg body weight oral vitamin E in combination with 1-100 mg/kg body weight R- or S-isomer of **alpha-lipoic acid**

DETD The general dose range of combinations with the above mentioned vitamins with R-**alpha-lipoic acid** for the antidiabetic effect one can use for example: 0.5-20 mg/kg body weight oral vitamin E in combination with 1-100 mg/kg body weight R- or S-isomer of **alpha-lipoic acid**

DETD . . . E in combination with 0.1 to 2000 mg, preferably 15 to 600 mg and in particular 50 to 200 mg R-**alpha-lipoic acid** or S-**alpha-lipoic acid**.

DETD . . . accordance with the invention, a daily dose of the combinations of the above named vitamins with the optical isomers of **alpha-lipoic acid** (R- or S-form) can be from 0.1 to 800 mg vitamin E, preferably 1 to 600 mg vitamin E in combination with the optical isomers of **alpha-lipoic acid** (R- or S-form in each case) 10-600 mg, preferably 25 to 400 mg or 10 to 200 mg. The maximum. . . treatment of states of pain and inflammation should not exceed 1.2 g for the racemate or R- or S-form of **alpha-lipoic acid** and 800 mg for vitamin E. The daily doses may be used in the form of a single administration of. . .

DETD . . . vitamin E in the combination and 0.1-20 mg/kg for the intramuscular administration of vitamin E in the combination) both for R-**alpha-lipoic acid** and also for S-**alpha-lipoic acid** is preferably 100 mg for the parenteral form of administration and 400 mg for the oral form.

DETD For example the daily dose for the parenteral form of administration of the R- or S-isomers of **alpha-lipoic acid** in the combination with the vitamin can in particular be 300 mg and 600 mg for the oral form.

DETD The medications are preferably given orally. For example, the vitamin E in the combination with R-**alpha-lipoic acid** and S-**alpha-lipoic acid** can in particular also be administered in the form of a solution, for example peroral, topical, parenteral (intravenous, intra-articular, intramuscular, subcutaneous), inhalative, transdermal. The medications containing as active substance for example vitamin E in combination with R-**alpha-lipoic acid** or S-**alpha-lipoic acid** can for example be formulated in the form of tablets, capsules, pills or coated tablets, granulates, pellets, plasters, solutions or. . . of pain and inflammatory states should for example for the combination of vitamin E with the R- or S-isomers of **alpha-lipoic acid** for the vitamin E not exceed 800 mg orally and for the R- and S-isomers of **alpha-lipoic acid** 1.2 g.

DETD . . . detoxifying, heavy metal antidote effect should for example for the combination of vitamin E with the R- or S-isomers of **alpha-lipoic acid** for the vitamin E not exceed 1200 mg orally and 1200 mg for the R- or S-isomers of **alpha-lipoic acid**.

DETD . . . example 800 mg orally, preferably 600 mg oral and parenteral

for vitamin E 15 mg/kg body weight intramuscular and for R-**alpha-lipoic acid** and also for S-**alpha-lipoic acid** preferably 80 mg for the parenteral form of administration and 200 mg for the oral form.

DETD The R-**alpha-lipoic acid** and S-**alpha-lipoic acid** in the combination with for example vitamin E can for example also be administered in particular in the form of. . . parenteral (intravenous, intra-articular, intramuscular, subcutaneous), inhalative, transdermal. Medicaments containing as active substances for example vitamin E in the combination with R-**alpha-lipoic acid** or S-**alpha-lipoic acid** can for example be formulated in the form of tablets, capsules, pills or coated tablets, granulates, pellets, plasters, solutions or. . . for example 0.5 to 20 weight %, preferably 1 to 10 weight % of one of the optical isomers of **alpha-lipoic acid** (in each case R-form or S-form) together with 0.001 to 10 weight % of the appropriate vitamin.

DETD The dosage unit of medications with for example vitamin E in combination with the optical isomers of **alpha-lipoic acid** or a therapeutically acceptable salt thereof (R-form or S-form in each case) can for example contain:

DETD . . . 10 to 1200 mg, preferably 20 to 600 mg, in particular 50 to 400 mg of the optical isomers of **alpha-lipoic acid** in combination with for example vitamin E 0.1 to 800 mg, preferably 1 to 400 mg, in particular 1-300 mg.

DETD . . . 1 to 4 times, in particular 1 to 3 times daily. However a total dose of the optical isomers of **alpha-lipoic acid** of 1200 mg and for example of vitamin E of 800 mg per day should not be exceeded for the. . . following medicinal forms listed under b) to e). In addition a total dose of the optical R- or S-isomers of **alpha-lipoic acid** of 2000 mg and for example of vitamin E of 1200 mg per day should not be exceeded for the. . .

DETD . . . 10 to 600 mg, preferably 15 to 500 mg, in particular 20 to 300 mg of the optical isomers of **alpha-lipoic acid** in the combination for example with vitamin E 0.01-20 mg/kg body weight intramuscular, preferably 0.1-12 mg/kg body weight in particular. . .

DETD . . . mucous membranes (for example as solutions, lotions, emulsions, ointments, plasters and the like) in the combination: 10 to 500 mg R-**alpha-lipoic acid** or S-alpha-lipoic 15 acid, preferably 40 to 250 mg, in particular 50 to 200 mg with for example the combination. . .

DETD . . . for inhalation (solutions or aerosols): 0.07 to 300 mg, preferably 0.25 to 150 mg, in particular 0.5 to 80 mg R-**alpha-lipoic acid** or S-**alpha-lipoic acid** combination with for example vitamin E preferably 0.001-20 mg/kg, in particular 0.01 to 10 mg/kg. These doses may for example. . .

DETD If solutions are used, the optical isomers of **alpha-lipoic acid** and the vitamins contained in the combination are preferably used in the form of a salt.

DETD . . . example 6 times the above stated dosage units. In particular tablets or capsules contain 20 to 800 mg of the **alpha-lipoic acid** or related compound in combination with a vitamin, for example vitamin E 1-1200 mg, pellets, powders or granulates 20 to 400 mg of the **alpha-lipoic acid** or

related compound in combination with a vitamin, for example vitamin E 1-800 mg, suppositories 20 to 300 mg of **alpha lipoic acid** or related compound in combination with a vitamin, for example 1-600 mg of vitamin E R-**alpha-lipoic acid** or S-**alpha-lipoic acid**.

DETD To combat retroviruses (for example AIDS) the daily dose is for example 4-6 g R- or S-isomer of **alpha-lipoic acid** in the combination with for example vitamin E 1-1200 mg.

DETD Corresponding medications consequently preferably contain in the combination with 5 mg-1 g vitamin E, R-**alpha-lipoic acid** or S-**alpha-lipoic acid** in the single dose (dosage unit) for example in an amount of 600 mg to 1.5 g.

DETD The above stated dosages always relate to combinations with the cited vitamins with, for example, the free optical isomers of **alpha-lipoic acid**. If the optical isomers of **alpha-lipoic acid** are used in the form of a salt, the stated dosages/dosage ranges should be increased accordingly on account of the. . .

DETD . . . event of the combination with the vitamins such as for example vitamin E being used with the optical isomers of **alpha-lipoic acid** in animals, the following indications may in particular be considered: panleucopenia, distemper, hepatoses, Arthrosis deformans, arthritis and dermatitis.

DETD . . . example possible to use the following dosages (vitamin E both in combination with the R-form and with the S-form of **alpha-lipoic acid**):

DETD For the treatment of cats, the oral single dose is generally between about 2 mg/kg and 50 mg/kg of the **alpha-lipoic acid** or related compound, in combination for example with vitamin E, 0.1 to 100 mg/kg, preferably 1 to 80 mg/kg, in particular 2-40 mg/kg body weight, the parenteral dose is between 0.5 and 40 mg/kg body weight of **alpha lipoic acid** or related compound in combination with the vitamin for example vitamin E 0.01 mg/kg to 10 mg/kg, preferably 0.1 to. . .

DETD For the treatment of arthroses in horses and cattle, the oral single dose in general in the combination for the **alpha-lipoic acid** or related compound is between about 2 mg/kg and 100 mg/kg body weight and for the vitamin between about 2 mg/kg and 100 mg/kg body weight, the parenteral dose in the combination for the **alpha-lipoic acid** or related compound is about between 0.5 and 50 mg/kg body weight and for the vitamin about between 0.005 and. . .

DETD The vitamin and **alpha-lipoic acid** or related compound such as the optical isomers of **alpha-lipoic acid** are suitable for the preparation of pharmaceutical compositions and formulations. The pharmaceutical compositions or medications contain for example the optical isomers of **alpha-lipoic acid** as active substance, optionally in a mixture with the vitamin or other pharmacological or pharmaceutically active substances. The medications are. . .

DETD The pharmaceutical and galenic handling of the vitamins and of the **alpha lipoic acid** or related compounds such as for example R- or S-**alpha-lipoic acid** is effected using conventional standard methods.

DETD . . . vitamin E, for example 250 mg dihydrolipoic acid, or in 10 ml vitamin E, for example 250 mg R-alpha or S-**alpha-lipoic acid**, and/or auxiliary or carrier substances are well mixed by stirring or homogenizing (for example using conventional mixing apparatus) (clear solution),. . .

DETD Administration of the vitamin with the **alpha-lipoic acid** or related compound, such as for example R- or S-**alpha-lipoic acid**, or of the medications can be to the skin or mucous membrane or to the inside of the body, for. . .

DETD If, for example, the vitamin E is used in combination with R- or S-**alpha-lipoic-acid** in the form of their

salts, the salt formers can also be used in excess, that is in a higher.

. .

DETD . . . It is also possible to use as complex formers those containing the vitamin in combination with for example R- or S-**alpha-lipoic acid** in a cavity.

DETD Antioxidants that may for example be used are sodium sulfite, sodium hydrogen sulfite, sodium metabisulfite, **ascorbic acid**, ascorbyl palmirate, -myristate, -stearate, gallic acid, gallic acid alkyl ester, butylhydroxyanisol, nordihydroguaiacic acid, **tocopherols** as well as synergists (substances which form heavy metals through complex formation, for example lecithin, **ascorbic acid**, phosphoric acid ethylene diamine tetraacetic acid, citrates, tartrates). The addition of synergists considerably raises the anti-oxygenic effect of the antioxidants.

DETD Suppositories with 50 mg dihydrolipoic acid or with R- or S-**alpha-lipoic acid** and 200 mg alphatocopherol or 200 mg alphatocopherol acetate

DETD . . . palmirate and 5 g Oxynex LM**) (E. Merck, Darmstadt) are suspended in 175 g molten hard fat*). 20 g alpha **tocopherol** and 5 g dihydrolipoic acid are then added thereto and the mixture is cast into hollow cells of 2.3 ml. . .

DETD . . . light brown to brown, waxy mass which melts on heating to 55.degree. C. to a clear brown liquid and contains **tocopherol**, ascorbyl palmirate, citric acid and lecithin.

DETD Suppositories with R- or S-**alpha-lipoic acid** may be prepared in the same manner by using the same amount of either R- or S-**alpha-lipoic acid** instead of dihydrolipoic acid.

DETD Capsules containing 200 mg dihydrolipoic acid or R- or S-**alpha-lipoic acid** and 500 mg alphatocopherol or alphatocopherol acetate

DETD 200 g R-**alpha-lipoic acid** are mixed with 500 g alphatocopherol. 595 g Miglyol.RTM. *) neutral oil and 100 g sorbitol syrup, 25 g glycerol. . . added thereto and the mixture filled into size 00 capsules. Each capsule weighing 1.42 g contains 200 mg R- or S-**alpha-lipoic acid** and 500 mg alphatocopherol.

DETD In the same manner it is possible to prepare capsules with dihydrolipoic acid or with S-**alpha-lipoic acid** by using the same amount of either dihydrolipoic acid or S-alphalipoic acid instead of R-**alpha-lipoic acid**.

DETD Ampoules containing 250 mg R- or S-**alpha-lipoic acid** and 250 mg vitamin C (**ascorbic acid**) in 10 ml

DETD 250 g R-**alpha-lipoic acid** are dissolved with stirring together with 352.3 tromethamine (2-amino-(hydroxymethyl)-1,3-propanediol) in a mixture of 8 liters of water sterilized for injection. . .

DETD Each ampoule contains 250 ml R-**alpha-lipoic acid** as tromethamine salt and 250 mg vitamin C in 10 ml injection solution.

DETD In the same manner it is possible to prepare ampoules with S-**alpha-lipoic acid** by using the same amount of S-**alpha-lipoic acid** instead of R-**alpha-lipoic acid**.

DETD Tablets with 50 mg S- or R-**alpha-lipoic acid** and 50 mg vitamin C **ascorbic acid**

DETD 250 g S-**alpha-lipoic acid** and 250 g vitamin C are evenly ground with 550 g microcrystalline cellulose. After sieving the mixture, 250 g starch. . .

DETD Each tablet contains 50 mg S-**alpha-lipoic acid** and 50 mg vitamin C.

DETD In the same manner it is possible to prepare tablets with 50 mg R-**alpha-lipoic acid** by using the same amount of R-**alpha-lipoic acid** instead of 250 g S-**alpha-lipoic acid.**

DETD Ampoules containing 50 mg dihydrolipoic acid or 50 mg R- or S-**alpha-lipoic acid** and 200 mg alphatocopherol acetate in 4 ml injection solution

DETD 50 g R-**alpha-lipoic acid** are dissolved with 750 g alphatocopherol acetate. The solution is diluted with 3200 g neutral oil.

DETD . . . filled in 10 ml portions into sterilized 10 ml ampoules under aseptic conditions. Each 10 ml ampoule contains 50 mg R-**alpha-lipoic acid** and 200 mg alphatocopherol acetate.

DETD In the same manner it is possible to prepare ampoules with dihydrolipoic acid or with S-**alpha-lipoic acid** by using the same amount of either dihydrolipoic acid or S-**alpha-lipoic acid** instead of R-**alpha-lipoic acid.**

DETD Ointment with 2% dihydrolipoic acid or 2% R- or S-**alpha-lipoic acid** and with 2% alphatocopherol

DETD 20 g R-**alpha-lipoic acid** are mixed with 20 g alphatocopherol with 400 g Vaselinum album and 100 g sorbitol 70% and 100 g Alcohol. . .

DETD The ointment contains 2% R-**alpha-lipoic acid** and 2% alphatocopherol acetate.

DETD In the same manner it is possible to prepare an ointment with dihydrolipoic acid or with S-**alpha-lipoic acid** by using the same amount of either dihydrolipoic acid-or S-**alpha-lipoic acid** instead of R-**alpha-lipoic acid.**

DETD Tablets containing 120 mg S- or R-**alpha-lipoic acid** and 61 mg vitamin C **ascorbic acid**

DETD 825 g S-**alpha-lipoic acid** and 425 g vitamin C are evenly ground with 550 g microcrystalline cellulose. After the mixture has been sieved, 250. . .

DETD Each tablet contains 120 mg S-**alpha-lipoic acid** and 61 mg vitamin C.

DETD In the same manner it is possible to prepare tablets with 120 mg R-**alpha-lipoic acid** by using the same amount of R-**alpha-lipoic acid** instead of S-**alpha-lipoic acid.**

CLM What is claimed is:

. . . to a mammal having diabetes mellitus Type I or Type II a therapeutically effective amount of a composition comprising pure R-**alpha-lipoic acid** and vitamin E, or a pharmaceutically acceptable salt thereof.

AN 96:99222 USPATFULL|

TI Combination medications containing **alpha-lipoic acid** and related|

IN Weischer, Carl-Heinrich, Bonn, Germany, Federal Republic of
Ulrich, Heinz, Niedernberg, Germany, Federal Republic of
Wessel, Klaus, Frankfurt, Germany, Federal Republic of

PA Asta Medica Aktiengesellschaft, Dresden, Germany, Federal Republic of (non-U.S. corporation)

PI US 5569670 19961029 <--

AI US 1995-404153 19950314 (8)

RLI Division of Ser. No. US 1994-197643, filed on 10 Feb 1994, now abandoned which is a continuation-in-part of Ser. No. US 1993-71259, filed on 4 Jun 1993, now abandoned

PRAI DE 1992-4218572 19920605

DT Utility|
EXNAM Primary Examiner: Dees, Jos e G.; Assistant Examiner: Lambkin, Deborah|
LREP Cushman Darby & Cushman, LLP|
CLMN Number of Claims: 1|
ECL Exemplary Claim: 1|
DRWN No Drawings
LN.CNT 1013|
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 64 OF 82 USPATFULL
PI US 5514517 19960507 <--
DETD . . . gelatin protective layer provided thereon is exposed and then developed in a processing solution of MAA-1 (i.e., abbreviation for metal **ascorbic acid** developer)+hypo 0.3 g/l at 20.degree. C. for 20 minutes.
DETD . . . mercaptobenzimidazoles, mercaptothiadiazoles, aminotriazoles, nitrobenzotriazoles, and benzotriazoles), mercaptopyrimidines, mercaptotriazines, thioketo compounds, azaindenes (e.g., triazaindenes, tetrazaindenes, pentazaindenes), benzenesulfonic acid, benzenesulfinic acid, benzenesulfonamide, .**alpha**.-**lipoic acid**, and derivatives of these compounds. Representative examples thereof include 1-phenyl-2-mercaptotetrazole, 4-hydroxy-6-methyl-1,3,3a,7-tetrazaindene, 2-mercaptobenzothiazole, and 5-carboxybutyl-1,2-dithiolan.
DETD . . . the hydrophilic binder for the photosensitive element of this invention. However, other hydrophilic binders are also usable. Examples thereof include **proteins** (e.g., gelatin derivatives, graft polymers of gelatin with other polymers, albumin, and casein), cellulose derivatives (e.g., hydroxyethyl cellulose, carboxymethyl cellulose,. . .
DETD . . . agent described above may be used in combination with Phenidone or a derivative thereof, p-aminophenol or a derivative thereof, or **ascorbic acid** as an auxiliary developing agent, and the combination with Phenidone or a derivative thereof is preferred.
AN 96:38744 USPATFULL
TI Process for image formation by silver salt diffusion transfer
IN Waki, Koukichi, Kanagawa, Japan
PA Fuji Photo Film Co., Ltd., Kanagawa, Japan (non-U.S. corporation)
PI US 5514517 19960507 <--
AI US 1995-370638 19950110 (8)
PRAI JP 1994-1768 19940112
DT Utility
EXNAM Primary Examiner: Schilling, Richard L.
LREP Sughrue, Mion, Zinn, Macpeak & Seas
CLMN Number of Claims: 11
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 1214
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 65 OF 82 USPATFULL
PI US 5508275 19960416 <--
SUMM . . . 1520), and the likewise lipid-soluble, but unstable, temperature- and light-sensitive vitamin E (ibid, No. 9832, page 1437) and the lipid-insoluble **ascorbic acid** (ibid, No. 846 page 120) are used as preservatives.
SUMM A.sub.5 --an **ascorbic acid** (derivative) radical ##STR4## in which E=O, S or NR.sup.9
SUMM . . . radicals of the type --O--, --S-- and/or --NR.sup.10 -- are separated from one another by at least 1 carbon or **phosphorus** atom;
SUMM where only 1 or 2 radicals R.sup.5 -R.sup.8 contain Q or are identical to Q (=an **ascorbic acid** radical).
SUMM . . . piperazine, mona-, di- and trierhanolamine,

ethyldiethanolamine, N-butylethanolamine,
tris(hydroxymethyl)aminomethan
e and the like. Suitable amine salts are, for example, those of
tryptamine, **cysteine** and the basic amine salts of lyeins and
arginine. Suitable quaternary ammonium cations are, for example,
tetramethylammonium and benzyltrimethylammonium. These cations can also
be used for salt formation of. . .

DETD . . . of BHT (=butylated hydroxytoluene) or 2,6-di-tert.-butyl-4-(7-
nonynoyl)-phenol [=compound according to Example 17] or ethyl
2-(3,5-di-tert.-butyl-4-hydroxybenzyl)-3-oxo-docosanoate [=compound
according to Example 18] or N-octadecyl-DL-.**alpha**.-
lipoic acid amide [=compound according to Example 52]
was added and the mixture was used in the customary manner as frying
fat.. . . probably comes about as a result of their lipophilic side
chains and, therefore, improved lipophilic interaction. The
advantageous
action of N-octadecyl-DL-.**alpha**.-**lipoic acid**
amide is particularly surprising, although this preparation has no
recognizable antioxidative component.

DETD . . .	<0.1	>50.0
Cpd. acc. to Ex. 27	>10.0	>1.0
Cpd. acc. to Ex. 28	<0.1	>50.0
Cpd. acc. to Ex. 29		
0.711	>1.0	>10.0
Ascorbic acid analog		
Ascorbic acid.sup.(1)		
2.99	>10.0	<0.1
Ascorbyl palmitate.sup.(1)	<0.1	>0.1
Cpd. acc. to Ex. 34		
0.052	<0.1	>1.0
Cpd. acc. to Ex. 35		
0.053	<0.1	>1.0

Cpd.. . .

CLM What is claimed is:

. . . atoms in the ring or the dithiol form of these radicals which has
been reduced by hydrogenation, and A.sub.5 --an **ascorbic**
acid radical or derivative thereof ##STR65## in which E is O, S
or NR.sup.9 R.sup.5 is H, EH, EQ or Q. . . the group consisting of
--O--, --S-- and --NR.sup.10 -- are separated from one another by at
least one carbon or **phosphorus** atom; and X is a lipophilic
component selected from the group consisting of X.sub.1 --a cholane
derivative radical, of the. . .

AN 96:31824 USPATFULL|

TI Lipid-selective antioxidants and their preparation and use|

IN Weithmann, Klaus-Ulrich, Hofheim am Taunus, Germany, Federal Republic
of
Wess, Gunther, Erlensee, Germany, Federal Republic of
Seiffge, Dirk, Mainz, Germany, Federal Republic of

PA Hoechst Aktiengesellschaft, Frankfurt am Main, Germany, Federal
Republic
of (non-U.S. corporation)

PI US 5508275 19960416

AI US 1994-212863 19940315 (8) <--

RLI Division of Ser. No. US 1991-638321, filed on 7 Jan 1991, now patented,
Pat. No. US 5318987

PRAI DE 1990-4000397 19900109

DT Utility|

EXNAM Primary Examiner: Ivy, C. Warren; Assistant Examiner: Owens, Amelia|

LREP Finnegan, Henderson, Farabow, Garrett & Dunner|

CLMN Number of Claims: 13|

ECL Exemplary Claim: 1|

DRWN No Drawings

LN.CNT 1144|

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 66 OF 82 USPATFULL

PI US 5472698 19951205 <--

SUMM . . . Fahim, U.S. Pat. No. 4,711,780 discloses a composition for epithelial regeneration containing vitamin C, a zinc salt and a sulfur **amino acid**, such as cystine or **cysteine**, or glutathione. Schreuder, U.S. Pat. 4,721,705 discloses the use of N-acyl **cysteine**, or S-acyl-**cysteine** in compositions for treating sun eczema or dishydrosis of skin. Several thiol compounds have been identified as effective UV light. . . Arch. Dermatol., Vol. 109, (April 1974) pp. 510-517, and WO 9404129 (Beiersdorf). Hillebrand, U.S. Pat. No. 5,296,500, discloses compositions containing N-acetyl-L-**cysteine** or derivatives thereof. The compositions are said to efface and prevent wrinkles in mammalian skin.

SUMM . . . of skin. Raaf, U.S. Pat. No. 4,743,442 and Pereira, U.S. Pat. No. 4,981,845 disclose skin care compositions which may contain **amino acids**, such as serine, cystine, **cysteine** . Ishida, U.S. Pat. No. 5,141,741, discloses anti-sunburn skin care compositions which may contain vitamins and **amino acids** as optional ingredients. **.alpha.-lipoic acid** is mentioned among suitable vitamins; serine and cystine are mentioned among suitable **amino acids**. Park, U.S. Pat. No. 5,135,741, discloses an anti-perspirant composition containing a compound having a basic nitrogen function, e.g., thiourea or **amino acids** such as serine.

SUMM The term "thiol" as employed herein means a compound containing at least one sulfhydryl group (--SH), other than the **amino acid** **cysteine**. The term "S-ester" as employed herein means a compound containing at least one group of formula ##STR1## wherein R is. . .

SUMM Examples of suitable thiol compounds include but are not limited tocystamine dihydrochloride cysteamine, N-acetyl-cysteamine, N-acetyl-L-**cysteine**, DL-6,8-thioctic acid (also known as DL.**alpha** .**-lipoic acid**) or salts thereof, thioacetamide, thioacetanilide, o-thiocresol, m-thiocresol, p-thiocresol, DL-6-thioctic acid, DL-6-thioctic amide, thiodiacetic acid, thiodiglycolic acid, thiosalicyclic acid, thiogalactoside, thiodiglucoside,. . .

SUMM Preferably, a thiol compound is N-acetyl-L-**cysteine** or . **alpha.-lipoic acid** because these two compounds are directly involved in natural skin-specific antioxidant pathways.

SUMM Suitable examples of compounds containing an S-ester group include but are not limited to **cysteine** S-esters (e.g., S-acetamidomethyl-L-**cysteine**, (S)-2-aminoethyl-L-**cysteine**, S-benzyl-L-**cysteine**, S-benzyl-L-**cysteine** ethyl ester, S-benzyl-L-**cysteine** methyl ester, S-t-butylmercapto-L-**cysteine**, S-carbamyl-L-**cysteine**, S-ethyl-L-**cysteine**, S-methyl-L-**cysteine**, S-lactoyl-**cysteine**, S-hydroxycaproyl-**cysteine**, S-adenosylmethionine, coenzyme A derivatives (e.g., N,S-Diacetyl-.beta.-mercaptoethylamine), S-esters of glutathione (e.g., S-lactoyl glutathione), S-butylglutathione, S-methylglutathione, S-decylglutathione, S-ethylgluta-thione, S-heptylglutathione, S-hexylglutathione, S-nonylglutathione, S-octyl-glutathione,. . .

SUMM . . . in combination with lipoic acid. In the second preferred embodiment, the inventive compositions contain serine or N-acetyl-serine in combination with N-acetyl-**cysteine** in order to attain maximum benefit and to minimize or substantially reduce the unpleasant odor.

DETD Materials: N-acetyl-L-**cysteine**, thioglycerin, thiosalicylic acid, **.alpha.-lipoic acid** N-acetyl-L-serine and L-serine were purchased from Sigma Chemical Co. St. Louis, Mo. Keratinocyte culture medium KGM and L-serine-free-KGM

were from. . .

DETD . . . keratinocytes were cultured in L-serine-free-KGM (37.degree. C,
5% CO.sub.2) to 85-95% of confluence for 4 days and then supplemented with N-acetyl-L-**cysteine** ("NAC") or **.alpha.-lipoic acid** at the concentrations listed in Tables 1 and 2 below. Cells so treated were then incubated for 24 hours under.

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.

DETD TABLE 2

Ceramide Production in the Presence of **.alpha.-Lipoic Acid**/L-Serine Combination

Test	Lipoic Acid Concentration (mM)	Serine Concentration (mM)	Ceramide Peak Area (arbitrary units)
A	0	0	0
B	0	10	0
C	0.1	0. . .	

DETD Similar enhancement was observed when **.alpha.-lipoic acid** was used as the thiol compound. Table 2 illustrates the effects of **.alpha.-lipoic acid** in combination with L-serine on the production of epidermal ceramides in cultured human keratinocytes. The results indicate that ceramide levels were undetectable in L-serine-free-KGM medium, or in KGM supplemented with 0.1 mM **.alpha.-lipoic acid** alone, or in KGM supplemented with 10 mM L-serine alone. High levels of ceramides were observed only when both **.alpha.-lipoic acid** and L-serine were provided to the cells.

DETD As indicated in Table 3, combination of **.alpha.-lipoic acid** (0.1 mM) and L-serine (0.6 mM) resulted in synergistic increase in.sup.3 H-serine incorporation into ceramide indicating an increase in the. . .

DETD . . . or S-esters on ceramide production in the cultured human keratinocytes. Cells were cultured as described in Example1 and treated with **.alpha.-lipoic acid** or S-lactoyl-glutathione at concentrations indicated in Table 5 below. Radioisotope labeled .sup.14 C-acetate (5 .mu.Ci/ml) was used to monitor the. . .

DETD The results in Table 5 indicate that both **.alpha.-lipoic acid** and s-lactoylglutathione at 2.0 mM concentration enhanced the production of ceramide more than three fold in a 24 hour period. . .

DETD . . . C., 5% CO.sub.2 over 5 days and treated with 2 mM thiol-supplemented DMEM. The thiols used in this experiment include N-acetyl-L-**cysteine** (NAC), thiosalicylic acid (TS), thioglycerin (TG), and mercaptosuccinic acid (MSA). The treated biopsies were metabolically labeled with .sup.3 H-serine (5. . .

DETD TABLE 6

Effect of Thiols on Ceramide Production

Test	L-Serine Concentration (mM)	Thiol (2 mM)	% Total Count
Control	0.4	None	9.31
A	0.4	N-acetyl-L-**cysteine**	10.59
B	0.4	Mercaptosuccinic acid	

			10.90
C	0.4	Thiosalycylic acid	
			12.25*
D	0.4	Thioglycerin	29.94*

*Statistically significant increase over control

DETD The lipid extract contains two layers. The lipid was subjected to the TLC analysis as described in Example 1. The **protein** assay on an aqueous layer was performed as follows:

DETD . . . described) was dried, under nitrogen, at 37.degree. C. 500 .mu.l (microliters) of 0.1 N NaOH was added to the dried **protein** extract which was mixed well to dissolve all of the **protein** present. 500 .mu.l of water was added to the extract to make the final NaOH concentration 0.05N.

DETD A series of **protein** standards (bovine serum albumin) was run along with the samples to create a standard curve.

DETD Comparison of samples to standards is performed by colorimetric assay using the Pierce Micro BCA **Protein** Assay Reagent Kit. (Pierce, cat# 23235).

DETD A color sensitive **protein** indicating solution (200 .mu.l/well) was added to each 50 .mu.l unknown (or standard) and, after a 30 minute incubation period,. . .

DETD After calculating the equation of the line of the standard curve, **protein** content was determined by plugging in absorbance figures and solving for **protein** concentration. This result was multiplied by 20 (since 50 .mu.l was taken from 1 ml) to determine the total **protein** content in the lipid extract's aqueous portion.)

DETD TABLE 7

Promotion of Ceramide Production by Lipoic Acid

Test	L-Serine Concentration (mM)	Lipoic Acid Concentration (mM)	ng ceramide/ .mu.g stratum corneum **protein**
Control			
	0.4	0	0.63
A	0.4	0.5	0.53
B	0.4	1.0	1.28*
C	0.4	2.0	2.29*

*Statistically significant increase over control

DETD TABLE 8

Promotion of Ceramide Production by N-acetyl-L-**cysteine**

Test	L-Serine Concentration (mM)	N-acetyl-L-**cysteine** Concentration (mM)	ng ceramide/ .mu.g stratum corneum **protein**
Control			
	0.4	0	0.63
A	0.4	0.2	2.29*
B	0.4	2.0	1.48*

*Statistically significant increase over control

DETD Example 2 was repeated except that the cells were treated with N-acetyl-L-serine and N-acetyl-L-**cysteine** as indicated in Table 9 below.

DETD TABLE 9

N-acetyl-L-Serine	N-acetyl-L-**cysteine**	.sup.3 H-serine

Test	Concentration (mM)	concentration (mM)	incorporation (% Total Count)
A	0	0	1.51
B	0	10	2.05
C	0.6	0	1.63
D	0.6. . .		

DETD The results in Table 9 indicate that the combination of N-acetyl-L-serine with N-acetyl-L-**cysteine** results in a synergistic increase in ceramide production.

DETD

	% w/w
L-serine	5
Fully hydrogenated coconut oil	3.9
N-acetyl **cysteine**	0.1
Brij 92*	5
Bentone 38	0.5
Preservative	0.3
MgSO.sub.4 7H.sub.2 O	0.3
Butyrated hydroxy toluene	0.01
Perfume	qs
Water	to 100

*Brij 92 is polyoxyethylene (2). . .

DETD

	% w/w
N-acetyl serine	5
Fully hydrogenated coconut oil	3.9
.alpha.-Lipoic acid	0.1
Brij 92*	5
Bentone 38	0.5
Preservative	0.3
MgSO.sub.4 7H.sub.2 O	0.3
Butyrated hydroxy toluene	0.01
Perfume	qs
Water	to 100

*Brij 92 is polyoxyethylene (2). . .

DETD

	% w/w
L-serine	10
Mineral oil	4
N-acetyl **cysteine**	1
Brij 56*	4
Alfol 16RD*	4
Triethanolamine	0.75
Butane-1,3-diol	3
Xanthan gum	0.3
Preservative	0.4
Perfume	qs
Butylated hydroxy toluene	0.01
Water	to 100

*Brij 56 is cetyl. . .

DETD

	% w/w

N-acetyl serine	10
Mineral oil	4
.alpha.-lipoic acid	1
Brij 56*	4
Alfol 16RD*	4
Triethanolamine	0.75
Butane-1,3-diol	3
Xanthan gum	0.3
Preservative	0.4
Perfume	qs
Butylated hydroxy toluene	0.01
Water	to 100

*Brij 56 is cetyl. . .

DETD

	% w/w
L-serine	1
N-acetyl **cysteine**	0.2
Ethanol	40
Perfume	qs
Butylated hydroxy toluene	0.01
Water	to 100

DETD

	% w/w
N-acetyl-L-serine	1
N-acetyl **cysteine**	0.2
Dimethylsulphoxide	10
Ethanol	40
Antioxidant	0.1
Perfume	qs
Water	to 100

DETD

	% w/w
N-acetyl serine	1
.alpha.-lipoic acid	0.2
Ethanol	40
Perfume	qs
Butylated hydroxy toluene	0.01
Water	to 100

DETD

	% w/w
L-serine	5
N-acetyl **Cysteine**	1
Ceramide-1	0.01
Silicone oil 200 cts	7.5
Glycerylmonostearate	3
Cetosteryl alcohol	1.6
Polyoxyethylene-(20)-cetyl alcohol	1.4
Xanthan gum	0.5
Parsol 1789	1.5
Octyl methoxycinnate (PARSOL MCX)	7
Perfume	qs
Color. . .	

DETD

	% w/w
N-acetyl serine	5
.alpha.-lipoic acid	1
Ceramide-1	0.01
Silicone oil 200 cts	7.5
Glycerylmonostearate	3
Cetosteryl alcohol	1.6
Polyoxyethylene-(20)-cetyl alcohol	1.4
Xanthan gum	0.5
Parsol 1789	1.5
Octyl methoxycinnate (PARSOL MCX)	7
Perfume	qs
Color. . .	

DETD

	% w/w
L-serine	5
N-acetyl **cysteine**	0.1
Silicone gum SE-30.sup.1	10
Silicone fluid 345.sup.2	20
Silicone fluid 344.sup.3	55.79
Squalene	10
Ceramides	0.01
Linoleic acid	0.01
Cholesterol	0.03
2-hydroxy-n-octanoic acid	0.7
Vitamin A palmitate	0.5
Vitamin. . .	

DETD

	% w/w
N-acetyl serine	5
.alpha.-lipoic acid	0.1
Silicone gum SE-30.sup.1	10
Silicone fluid 345.sup.2	20
Silicone fluid 344.sup.3	55.79
Squalene	10
Ceramides	0.01
Linoleic acid	0.01
Cholesterol	0.03
2-hydroxy-n-octanoic acid	0.7
Vitamin A palmitate	0.5
Vitamin. . .	

CLM What is claimed is:

. . . N-acetyl-L-serine and mixtures thereof; (ii) from about 0.0001% to about 50% of an ingredient selected from the group consisting of N-acetyl-**cysteine**, **.alpha.-lipoic acid** and mixtures thereof; and (iii) a cosmetically acceptable vehicle.

4. The composition of claim 1 wherein ingredient (i) is N-acetyl serine and ingredient (ii) is **.alpha.-lipoic acid** .

5. The composition of claim 1 wherein ingredient (i) is selected from the group consisting of serine and N-acetyl serine, and ingredient (ii) is N-acetyl-**cysteine**.

. . . N-acetyl-L-serine and mixtures thereof; (ii) from about 0.0001% to about 50% of an ingredient selected from the group consisting of N-acetyl-**cysteine**, **.alpha.-lipoic acid**, S-lactoyl-glutathione and mixtures thereof; and (iii) a cosmetically acceptable vehicle.

9. The method of claim 6 wherein ingredient (i) is N-acetyl serine and ingredient (ii) is **.alpha.-lipoic acid**.

10. The method of claim 6 wherein ingredient (i) is selected from the group consisting of serine and N-acetyl serine, and ingredient (ii) is N-acetyl-**cysteine**.

AN 95:107924 USPATFULL|
TI Composition for enhancing lipid production in skin|
IN Rawlings, Anthony V., Wyckoff, NJ, United States
Zhang, Kelly H., Piscataway, NJ, United States
Kosturko, Richard, Nutley, NJ, United States
PA Elizabeth Arden Co., Division of Conopco, Inc., New York, NY, United States (U.S. corporation)
PI US 5472698 19951205
AI US 1994-359758 19941220 (8) <--
DT Utility|
EXNAM Primary Examiner: Kishore, Gollamudi S.|
LREP Mitelman, Rimma|
CLMN Number of Claims: 10|
ECL Exemplary Claim: 1|
DRWN No Drawings
LN.CNT 908|
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 67 OF 82 USPATFULL
PI US 5411991 19950502 <--
SUMM Hair **proteins** include a fairly large quantity of the **amino acid cysteine**, which includes a thiol (--SH) group. It is the formation of disulfide bonds between **cysteine** residues in the hair **proteins**, to form cystine, that give hair its strength and character.
SUMM . . . active compounds reduce hair growth at least in part by one or more of the following mechanisms. During hair growth, **cysteine** is incorporated into **protein** chains. The --SH groups of **cysteine** residues in the **protein** chains form disulfide bonds (and cystine), binding the **protein** chains together as part of the normal hair growth. Sulfhydryl active compounds, applied topically, penetrate the hair follicle and interfere with hair growth by

(1) reacting with free **cysteine** to form a mixed **cysteine**-sulfhydryl active compound disulfide bond, resulting in there being less **cysteine** available for incorporation into disulfide bonds present in hair **proteins**; (2) reducing the disulfide bond in cystine in the hair **proteins**, at the same time forming a mixed **cysteine**-sulfhydryl active compound disulfide bond; and (3) reducing the disulfide bond in cystine, without concomitant formation of the mixed disulfide bond.
SUMM Preferred sulfhydryl active compounds with a free --SH group include thiosalicylic acid, D-**cysteine**, 2-mercaptoethylamine (cysteamine), captopril, N-acetyl-L-**cysteine**, cysteinylglycine, 2,3-dimercapto-1-propanesulfonic acid, meso-2,3-dimercaptosuccinic acid, dimethylcysteamine, diethyldithiocarbamic acid, D-penicillamine, L-**cysteine** methyl ester, and L-**cysteine** ethyl ester.
SUMM . . . a free --SH group include 3,3'-thiodipropionic acid, isethionic

acid, 3-carboxypropyl disulfide, 3,3'-thiodipropionic acid dilauryl ester, sulfasalazine, 3-(methythio)-propylamine, 5'-deoxy-5'-methylthioadenosine, allyl sulfide, DL-.**alpha.-lipoic acid** (reduced form), and DL-methionine-S-methyl-sulfonium chloride.

SUMM . . . include phosphocysteamine, which is dephosphorylated to cysteamine in cells; penicillamine disulfide, which is reduced to free penicillamine in cells; and S-2-aminoethyl-L-**cysteine**, which is hydrolyzed to cysteamine and serine (inactive) in cells.

DETD

. . . 0.18 .+-. 0.04
1.64 .+-. 0.04
89 .+-. 2%

2-Mercaptoethylamine (Cysteamine)
20%
A 0.30 .+-. 0.09
1.89 .+-. 0.34
86 .+-. 3%

L-**Cysteine** methyl ester
20%
A 0.28 .+-. 0.07
1.91 .+-. 0.30
86 .+-. 3%

L-**Cysteine** ethyl ester
20%
A 0.49 .+-. 0.08
2.73 .+-. 0.15
82 .+-. 3%

N-Acetyl-L-**Cysteine**
15%
A 0.39 .+-. 0.07
2.13 .+-. 0.31
80 .+-. 4%

2,3,-Dimercapto-1-propanesulfonic acid
20%
A 0.64 .+-. 0.08
3.08 .+-. 0.27

. . . A 0.57 .+-. 0.07
1.87 .+-. 0.3
65 .+-. 5%

Sulfasalazine 20%
C 0.88 .+-. 0.14
2.32 .+-. 0.21
61 .+-. 6%

D-**Cysteine** 10%
A 1.20 .+-. 0.17
2.92 .+-. 0.24
60 .+-. 3%

5'-Deoxy-5'-methylthioadenosine
10%
A 1.25 .+-. 0.17
2.97 .+-. 0.27
57 .+-. 6%

Captopril 10%
A 1.49 .+-. 0.20
3.50 .+-. 0.15
57 .+-. 5%

DL-.**alpha.-Lipoic acid** (reduced form)
15%
A 0.74 .+-. 0.09
1.73 .+-. 0.19
56 .+-. 6%

Cystenyl-glycine 15%
A 0.93 .+-. 0.18
2.26 .+-.. . . 0.16
2.23 .+-. 0.28
50 .+-. 5%

3,3'-Thiodipropionic acid dilauryl ester
20%
D 1.07 .+-. 0.10
2.15 .+-. 0.08
50 .+-. 4%

S-2-Aminoethyl-L-**cysteine**
20%
A 0.99 .+-. 0.20
2.15 .+-. 0.35
50 .+-. 11%

3,3'-Thiodipropionic acid dilauryl ester
5%
D 1.70 .+-. 0.21
2.39. . .

DETD The following biochemical properties of some of the sulfhydryl reactive compounds were tested: (1) the percent reduction in hair shaft **cysteine** caused by the compounds; (2) the ability of the compounds to form a **cysteine**-mixed disulfide in vitro; (3) the ability of the compound to form a **cysteine**-mixed disulfide in hair shafts; and (4) the ability of the compounds to reduce cystine.

DETD The percent reduction in hair shaft **cysteine** caused by the sulfhydryl reactive compounds was measured according to the following procedure. **Amino acid** analysis of hamster flank organ hairs was carried out using a commercially available **amino acid** analysis system (Pico-Tag system, available from Waters Associates, Inc., Milford, Mass.). The hairs were thoroughly washed, then hydrolyzed by HCL vapors at 115.degree. C. overnight. The hydrolyzed hairs (now free **amino acids**) were derivatized with phenylisothiocyanate to yield the phenylthiohydantion derivatives of the respective **amino acids**, which were then separated by C-18 reverse phase chromatography (HPLC), and quantitated by an in-line UV spectrophotometer. It is believed that the reduction of **cysteine** levels in hair shafts caused by some of the sulfhydryl active compounds is at least in part responsible for the.

. .

DETD The ability of the sulfhydryl reactive compounds to form **cysteine**-mixed disulfides in hair shafts was determined according to the following procedure. Groups of eight (8) Golden Syrian hamsters were treated. . . treatments (Mon-Fri, over 18 days), hair shafts from the treated flank organs were harvested and analyzed for the

presence of **cysteine**-mixed disulphides. It is believed that the ability of some of the sulfhydryl reactive compounds to form the cystein-emixed disulfides in. . . is at least in part responsible for

the reduction in hair growth caused by these compounds, as the hair shaft **proteins** fail to undergo final post-translational maturation (disulfide formation).

DETD The ability of the sulfhydryl reactive compounds to form **cysteine**-mixed disulfides in vitro was determined by incubating the sulfhydryl reactive compounds in test tubes, with either cystine or **cysteine**, under physiological conditions (i.e. pH 7.4 and at a temperature of 37.degree. C.). The reaction of these compounds with **cysteine** or cystine was evaluated by HPLC analysis. It is believed that the ability of a sulfhydryl reactive compound to form a **cysteine**-mixed disulfide in vitro provides an indication that the compound is capable of forming **cysteine**-mixed disulfides with free **cysteine** present in hair follicle bulbs prior to **cysteine** incorporation into **protein** of the hair shaft when applied topically to the skin.

DETD . . . phosphocysteamine and dimethylcysteamine the samples were analyzed without derivatization, using an electrochemical detector instead of the UV detector used in **amino acid** analysis. The determination of cystine reduction by the compounds was based on generation of **cysteine** (free thiol) in the incubation

mixture. It is believed that reducing the disulfide bond in cystine in hair **proteins** results in reduced hair growth.

DETD TABLE 2

Biochemical Properties of Select Sulfhydryl Reactive Agents

Sulfhydryl reactive agent	Percent reduction in hair shaft **cysteine**	Formation of **Cysteine** mixed disulfide in-vitro in hair shaft	Reduction of Cystine
D-Penicillamine	50%	YES	YES	ND*
Cysteamine	50%	YES	YES	YES
Dimethyl cysteamine	28%	YES	YES	YES
Phospho cysteamine				

. . .

CLM What is claimed is:

2. The process of claim 1 wherein said compound reacts with free **cysteine** in hair follicle cells to form **cysteine**-mixed disulfides.

3. The process of claim 1 wherein said sulfhydryl active compound reduces disulfide bonds in cystine in hair **proteins.**

4. The process of claim 3 wherein said sulfhydryl active compound also forms a mixed disulfide bond with one of the **cysteine** moieties in hair shaft **proteins.**

12. The process of claim 1 wherein said sulfhydryl active compound is D-**cysteine.**

13. The process of claim 1 wherein said sulfhydryl active compound is N-acetyl-**cysteine.**

17. The process of claim 1 wherein said sulfhydryl active compound is L-**cysteine** methyl ester.

19. The process of claim 1 wherein said sulfhydryl active compound is L-**cysteine** ethyl ester.

AN 95:38702 USPATFULL|
TI Method of reducing hair growth employing sulfhydryl active compounds|
IN Shander, Douglas, 16112 Howard Landing Dr., Gaithersburg, MD, United States 20878
Ahluwalia, Gurpreet S., 8632 Stable View Ct., Gaithersburg, MD, United States 20879
Grosso, Diana M-D., 4513 W. Brook La., Keningston, MD, United States 20895
PI US 5411991 19950502 <--
AI US 1992-995037 19921222 (7)
DT Utility|
EXNAM Primary Examiner: Henley, III, Raymond; Assistant Examiner: Moezle, M.|
LREP Fish & Richardson|
CLMN Number of Claims: 31|
ECL Exemplary Claim: 1|
DRWN No Drawings
LN.CNT 428|
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 68 OF 82 USPATFULL

PI US 5334612 19940802

AB . . . hydrogen or C.sub.1 -C.sub.6 alkyl and n represents a number from 1 to 10, or their pharmaceutically acceptable salts including . **alpha.-lipoic acid** and dihydrolipoic acid. <-- The pharmaceutical compositions are useful for the treatment of diseases caused by retroviruses.

SUMM . . . -C.sub.6 -alkyl and n represents a number from 1 to 10, or their pharmaceutically acceptable salts with the exception of . **alpha.-lipoic acid** and dihydrolipoic acid. The dosage unit for solid or semi-solid formulations of these compounds contain 20 mg to 6 g, . . .

SUMM B. Pharmaceutical compositions containing . **alpha.-lipoic acid** in a dosage unit for solid or semi-solid formulations which contains 51 mg to 6 g, in particular 100 mg. . . to 2 g, preferably 200 mg to 1 g or also 400 mg or 500 mg to 1 g of . **alpha.-lipoic acid** or a pharmaceutically acceptable salt thereof, or in the form of injection solutions which contain 26 mg to 500 mg. . . per ml, preferably 1 mg to 50 mg per ml, in particular 5 mg to 10 mg per ml of . **alpha.-lipoic acid** or a pharmaceutically acceptable salt thereof.

SUMM . . . containing one, two or three hydroxyl groups, polyethylene glycols of molecular weights between 200-600; conventional physiologically acceptable organic amides, natural .alpha.-**amino acids**, aliphatic amines, hydroxyethyl theophylline, tromethamine, diethylene glycol monomethyl ether.

SUMM . . . -C.sub.6 -alkyl and n represents a number from 1 to 10 or their therapeutically acceptable salts with the exception of . **alpha .-lipoic acid** and dihydrolipoic acid along with conventional pharmaceutical carriers and/or diluents or other auxiliary substances which comprises processing into pharmaceutical formulations. . .

SUMM . . . C.sub.1 -C.sub.6 -alkyl and n represents a number from 1 to 10 their therapeutically acceptable salts with the exception of . **alpha.-lipoic acid** and dihydrolipoic acid is processed with conventional pharmaceutical carrier substances and/or diluting agents or other auxiliary substances into pharmaceutical formulations. . .

SUMM G. A process for the preparation of a pharmaceutical composition in which . **alpha.-lipoic acid** or dihydrolipoic acid is processed into pharmaceutical formulations or brought into a therapeutically acceptable form with conventional pharmaceutical carrier substances. . . and/or diluting agents or other auxiliary substances, the dosage unit for solid or semi-solid formulations in the case of the . **alpha.-lipoic acid** containing 51 mg to 6 g, preferably 100 mg to 2 g, in particular 200 mg to 1 g or also 400 mg or 500 mg to 1 g of . **alpha.-lipoic acid** or a pharmaceutically acceptable salt thereof or, in the case of the . **alpha.-lipoic acid**, injection solutions that contain 26 mg to 500 mg per ml, preferably 50 mg to 200 mg, in particular 100 mg per ml of . **alpha.-lipoic acid** or a pharmaceutically acceptable salt thereof, or drinkable solutions, suspensions or emulsions which contain 0.2 mg to 500 mg per ml, preferably 1 mg to 50 mg per ml, in particular 5 mg to 10 mg per ml of . **alpha.-lipoic acid** or a pharmaceutically acceptable salt thereof, the dosage unit for solid or semi-solid formulations in the case of the dihydrolipoic. . .

SUMM a) in the case of compounds of Formula I with the exception of . **alpha.-lipoic acid** and dihydrolipoic acid, containing 20 mg to 6 g, in particular 50 mg to 2 g, preferably 100 mg to. . .

SUMM b) in the case of . **alpha.-lipoic acid**,

containing 51 mg to 6 g, in particular 100 mg to 2 g, preferably 200 mg to 1 g or also 400 mg or 500 mg to 1 g of **.alpha.-lipoic acid** or a salt thereof;

SUMM . . . in particular 0.5 to 20, preferably 1 to 10% by weight of the total amount of active substance I (including **.alpha.-lipoic acid**) or of a salt thereof;

SUMM a) in the case of compounds of Formula I with the exception of **.alpha.-lipoic acid** and dihydrolipoic acid contains 0.2 mg to 800 mg per ml, in particular 10 mg to 500 mg, preferably 40. . .

SUMM b) in the case of injection solutions of **.alpha.-lipoic acid**, contains 26 mg to 500 mg per ml, in particular 50 mg to 200 mg, preferably 100 mg per ml **.alpha.-lipoic acid** or of a salt thereof;

SUMM c) in the case of drinkable solutions, suspensions or emulsions of **.alpha.-lipoic acid**, contains 0.2 mg to 500 mg per ml, preferably 1 mg to 50 mg per ml, in particular 5 mg to 10 mg per ml **.alpha.-lipoic acid** or a pharmaceutically acceptable salt thereof;

SUMM . . . contain one, two or three hydroxyl groups, polyethylene glycols with molecular weights between 200-600; conventional physiologically acceptable organic amides, natural .alpha.-**amino acids**, aliphatic amines, hydroxyethyl theophylline, tromethamine, diethylene glycol monomethyl ether.

SUMM The compounds of Formula I including **.alpha.-lipoic acid** and dihydrolipoic acid may also be used in the form of their optical isomeric (R(+) and S-(-) form, compounds of. . . for the preparation of pharmaceutical compositions and formulations and for the cited use. The compounds of Formula I are preferably **.alpha.-lipoic acid** and dihydrolipoic acid (racemates as well as the corresponding enantiomers).

SUMM **.alpha.-lipoic acid** is widely available in the form of the racemate (Thioctsaure.sup.R) in plants and animals; it acts as co-enzyme in many. . . reactions, constitutes a growth factor for certain bacteria and protozoas and is used in death-head fungus poisoning. In addition, the **.alpha.-lipoic acid** racemate displays anti-inflammatory, antinociceptive (analgesic) and cytoprotective properties.

SUMM In the hitherto used formulations, the **.alpha.-lipoic acid** and dihydrolipoic acid are present in relatively small amounts.

SUMM The pharmaceutical compositions of the invention which contain larger amounts of **.alpha.-lipoic acid** and dihydrolipoic acid are novel; moreover the fact that higher dosages of these active substances display more advantageous pharmaceutical effects,. . .

SUMM . . . which are physiologically acceptable in the salt form. Examples thereof are: alkali metals or alkaline earth metals, ammonium hydroxide, basic **amino acids** such as **arginine** and lysine, amines of formula NR.sub.1 R.sub.2 R.sub.3 where the radicals R.sub.1, R.sub.2 and R.sub.3 are the same or different. . . or hexamethylene tetramine, saturated cyclic amino compounds with 4-6 cyclic carbon atoms such as piperidine, piperazine, pyrrolidine, morpholine; N-methyl glucamine, **creatine**, tromethamine.

SUMM . . . with 2-6 carbon atoms, such as ethylene diamine, hydroxyethyl theophylline, tromethamine (for example as 0.1 to 20% aqueous solution), aliphatic **amino acids**. The **amino acids** are for example **amino acids** having the following structure: ##STR3## where R' represents hydrogen, a phenyl radical, an indolyl-(3)-methyl radical, imidazolyl-(4)-methyl radical, a

C.sub.1 -C.sub.10. . .

SUMM

lipoic acid	10%
L-lysine	7.66%
ethylene diamine	0.27%
water	82.07%
.alpha.-lipoic acid	
	10%
L-lysine	7.66%
tromethamine	1%
water	81.34%
dihydrolipoic acid	
	1%
tromethamine	0.9%
ethylene diamine	0.38%
water	97.72%
dihydrolipoic acid	
	1%
tromethamine	1.5%
1,2-propylene glycol	
	20%
nicotinic acid amide	
	10%
water	67.5%

SUMM The complex formers used may also be those enclosing the R- or S- . **alpha.-lipoic acid** in a hollow space.
Examples thereof are urea, thiourea, cyclodextrines, amylose.

SUMM Antioxidants that may for example be used are sodium sulphite, sodium hydrogen sulphite, sodium metabisulphite, **ascorbic acid**, ascorbylpalmitate, -myristate, -stearate, gallic acid, gallic acid alkyl ester, butylhydroxyanisol, nordihydroguaiacic acid, **tocopherols** as well as synergists (substances which bind heavy metals through complex formation, for example lecithin, **ascorbic acid**, phosphoric acid ethylene diamine tetraacetic acid, citrates, tartrates). Addition of synergists substantially increases the antioxygenic effect of the antioxidants.

SUMM Thus, for example, a single dose of 0.035 mg/ml of compound I (for example **alpha-lipoic acid**, racemate) reduces the number of infectious viruses (for example HIV-1) in cell culture supernatant from 100% in the positive control. . .

SUMM Cell type: human epithelium-like. Growth medium: culture medium consisting of various **amino acids** and electrolytes for the cultivation of epithelial cells (for example Dulbecco's minimal essential medium, DME), 90%; serum of newborn calves,. . .

SUMM FURTHER INFORMATION ON HIV REPLICATION BY **.alpha.-LIPOIC ACID** IN VITRO

SUMM . . . (PFU). The tumor cell line permanently infected in this manner (Molt4) is treated for three weeks with 70 .mu.g of **.alpha.-lipoic acid**/ml. Every three days culture medium and **alpha.-lipoic acid** are replaced and the activity of the virus determined in the reverse transcriptase and plaque test. The reverse transcriptase test. . .

SUMM . . . from day 6 and reaches a reduction of 90% after three weeks. These results demonstrate the great antiviral potency of **.alpha .-lipoic acid**. The second important result worthy of recording is that there is no sign or evidence of the development of tolerance. . .

SUMM Should one wish to compare the in vitro effect of **.alpha.-lipoic acid** with other agents which have already been successfully used in the treatment of AIDS, alpha interferon may be considered. The. . . i.e. an inhibitory effect on the so-called budding or ejection process of the virus has been discussed. In common with **.alpha.-lipoic acid**, alpha interferon

therefore acts on the already infected cell. To compare both compounds, freshly split Jurkat cells were infected with HIV (8.times.10.sup.3 PFU)

and afterwards recombinant alpha interferon (rIF) (70 units/ml) or 35 ng/ml of **.alpha.-lipoic acid** was added
thereto using a pipette. The experiment was concluded after 7 days in order to assess in particular the. . . FIG. 2), the inhibition in the

plaque test (indicates the exact number of infectious virions, FIG. 3) is clearer for **.alpha.-lipoic acid**. The
combination of both substances shows an additive effect.

SUMM . . . action. AZT inhibits reverse transcriptase and thus acts predominantly on non-infected cells. However, once a cell has been infected, AZT--unlike **.alpha.-lipoic acid**
--is no longer able to inhibit the growth of the virus.

SUMM 3. **.alpha.-lipoic acid** is added using a
pipette.

SUMM INHIBITION OF HIV REPLICATION BY **.alpha.-LIPOIC ACID** IN VIVO METHOD

SUMM Determination of the plasma p24 antigen level using a commercial ELISA; p24 is the designation for a structural **protein** of the HIV
virus; ELISA (Enzyme Linked Immuno Sorbent Assay) is a test technique frequently used in virology in order to determine **proteins**, antigens, etc.

SUMM The following results were recorded in 4 patients in Walter Reed stage 6. Application of **.alpha.-lipoic acid** was,
by means of infusion of an **.alpha.-lipoic acid** solution having the following composition: 10 ml of aqueous solution containing 250 mg of **.alpha.-lipoic acid** in the form of the ethylene diamine salt (=323 mg salt) as well as 1 g of 1,2-propylene glycol and. . .

SUMM The infusion of **.alpha.-lipoic acid** was
given through a central venous catheter over 24 hours. Because of initially non-excluded interactions with Zovirax (on account of Herpes Zoster) the **.alpha.-lipoic acid** infusion
was interrupted during the administration of Zovirax (3 times 1-2 hours each/day).

SUMM Total dose of **.alpha.-lipoic acid** of 104 g
on 26 days during the 27-day therapy phase. The dosage administered was:

on 2 days 2 g/day,. . .

SUMM Therapy phase 19 days. Total dose of **.alpha.-lipoic acid**: 82.75 g. Therapy as from 22.05.1990 with **.alpha.-lipoic acid** permanent infusion for 20-24
hours/day. Doses: 2 days 2 g, 9 days 4 g, 1 day 3 g, 1 day. . .

SUMM . . . in these parameters and thus that a positive effect on the entire symptomatology may be expected with longer-term treatment with **.alpha.-lipoic acid.**

SUMM For the combination of active ingredients of Formula I (for example **.alpha.-lipoic acid**) with component b, for
example AZT, the two components may in each case be mixed in a ratio of 1. . .

SUMM In the event of a combination of active substances of Formula I (for example **.alpha.-lipoic acid**) and
.alpha.-interferon, the two components may for example be present in the

following ratio: 50 mg-6 g of compound I. . .

SUMM The acute toxicity of **.alpha.-lipoic acid**
in the mouse (expressed in the LD 50 mg/kg; Method after Miller and Tainter: Proc. Soc. Exper. Biol. a. Med.. . .

DETD Tablets containing 50 mg S- or R- **.alpha.-lipoic acid**

DETD 250 g S- **.alpha.-lipoic acid** are evenly
ground with 750 g microcrystalline cellulose. After sieving the mixture,
250 g starch (starch 1500/ Colorcon), 732.5 g. . .

DETD Each tablet contains 50 mg S- **.alpha.-lipoic acid.**

DETD In the same way it is possible to prepare tablets with 50 mg R- **.alpha.-lipoic acid** by using the same amount of R-**.alpha.-lipoic acid** instead of 250 g of S- **.alpha.-lipoic acid.**

DETD Ampoules containing 50 mg S- or R- **.alpha.-lipoic acid** as tromethamine salt in 2 ml

DETD 250 g S- **.alpha.-lipoic acid** are dissolved together with 352.3 g tromethamine (2-amino-2-(hydroxymethyl)-1,3-dihydroxypropane) in a mixture of 9 liters of water for injection purposes and. . .

DETD Each ampoule contains 50 mg S- **.alpha.-lipoic acid** as tromethamine salt in 2 ml injection solution.

DETD In the same way it is possible to prepare ampoules with R- **.alpha.-lipoic acid** by using the same amount of R- **.alpha.-lipoic acid** instead of 250 g S- **.alpha.-lipoic acid.**

DETD FIG. 1 is a graph showing reductions in plaque count and reverse transcriptase count caused by **.alpha.-lipoic acid**, as a function of time;

DETD FIG. 2 is a graph showing reductions in reverse transcriptase count caused by **.alpha.-lipoic acid**, recombinant .alpha.-interferon and a combination of **.alpha.-lipoic acid** and recombinant .alpha.-interferon; and

DETD FIG. 3 is a graph showing reductions in plaque count caused by **.alpha.-lipoic acid**, recombinant .alpha.-interferon and a combination of **.alpha.-lipoic acid** and .alpha.-interferon.

CLM What is claimed is:

. . . is hydrogen or a C.sub.1 -C.sub.6 -alkyl and n represents a number from 1 to 10 with the exception to **.alpha.-lipoic acid** and dihydrolipoic acid, or a pharmaceutically acceptable salt of said compound of Formula I the dosage unit being a solid. . .

6. A dosage unit of a pharmaceutical composition containing **.alpha.-lipoic acid**, the dosage unit being a solid or semi-solid formulation containing 51 mg to 6 g **.alpha.-lipoic acid** or a pharmaceutically acceptable salt thereof or an injection solution which contains 26 mg to 500 mg per ml of **.alpha.-lipoic acid** or a pharmaceutically acceptable salt thereof, or a drinkable solution, suspension or emulsion which contains 1 mg to 500 mg per ml of **.alpha.-lipoic acid** or a pharmaceutically acceptable salt thereof.

. . . unit of a solid or semi-solid pharmaceutical composition as set forth in claim 6 containing 100 mg to 2 g **.alpha.-lipoic acid** or a pharmaceutically acceptable salt thereof.

. . . unit of a solid or semi-solid pharmaceutical composition as set forth in claim 6 containing 200 mg to 1 g **.alpha.-lipoic acid** or a pharmaceutically acceptable salt thereof.

9. An injection solution as set forth in claim 6 which contains 50 mg to 200 mg per ml **.alpha.-lipoic acid** or a pharmaceutically acceptable salt thereof,

10. An injection solution as set forth in claim 6 which contains 100 mg per ml of **.alpha.-lipoic acid** or a pharmaceutically acceptable salt thereof.

. . . solution, suspension or emulsion as set forth in claim 6 which

contains 1 mg to 50 mg per ml of .**alpha**.-**lipoic** **acid** or a pharmaceutically acceptable carrier thereof.

. . . solution, suspension or emulsion as set forth in claim 6 which contains 5 mg to 10 mg per ml of .**alpha**.-**lipoic** **acid** or a pharmaceutically acceptable salt thereof and a liquid, pharmaceutically acceptable carrier therefor.

AN 94:66503 USPATFULL|
TI Pharmaceutical compositions containing as active substance sulphur-containing carboxylic acids and their use in combating retroviruses|
IN Kalden, Joachim, Erlangen, Germany, Federal Republic of
Fleckenstein, Bernhard, Wiesenthau, Germany, Federal Republic of
Baur, Andreas, Erlangen, Germany, Federal Republic of
Harrer, Thomas, Erlangen, Germany, Federal Republic of
PA ASTA Pharma Aktiengesellschaft, Frankfurt, Germany, Federal Republic of (non-U.S. corporation)
PI US 5334612 19940802 <--
AI US 1990-610560 19901108 (7)
PRAI DE 1988-3937325 19881109
DE 1990-4015728 19900516
DT Utility|
EXNAM Primary Examiner: Nutter, Nathan M.|
LREP Cushman, Darby & Cushman|
CLMN Number of Claims: 22|
ECL Exemplary Claim: 1|
DRWN 3 Drawing Figure(s); 3 Drawing Page(s)|
LN.CNT 1085|
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 69 OF 82 USPATFULL
PI US 5324656 19940628 <--
SUMM . . . DME, include M199 (Askanas, V. and Engel, W. K. (1975) Neurology 25:58-67; Konigsberg, I. R. (1963) supra), MEM plus nonessential **amino acids**, pyruvate and additional vitamins (Miranda, A. F. et al. (1979) in Muscle regeneration, Mauro, S.
et al. (eds.), Raven Press,. . .
SUMM . . . the composition of MCDB 120. Clonal growth of HMSC was sensitive in particular to the concentrations of the medium components L-**arginine**, L-methionine, L-threonine, D-pathothenic acid and myo-**inositol**. It is believed that MCDB 120 is the first optimized basal nutrient medium that has been developed specifically for
the. . .
SUMM TABLE 1

Composition of MCDB 120 and MCDB 131M

	MCDB 120 moles/liter	MCDB 131M.sup.1 moles/liter
AMINO ACIDS		
L-**Alanine**	3 .times. 10.sup.-5	
L-**Arginine**.HCl	1 .times. 10.sup.-3	3 .times. 10.sup.-4
L-Asparagine.H.sub.2 O	1 .times. 10.sup.-4	
L-Aspartic Acid	1 .times. 10.sup.-4	
L-**Cysteine**.HCl.H.sub.2 O	2 .times. 10.sup.-4	
L-Glutamic Acid	3 .times. 10.sup.-5	
L-**Glutamine**	1 .times. 10.sup.-2	
Glycine	3 .times. 10.sup.-5	
L-Histidine.HCl.H.sub.2 O		

	2 .times. 10.sup.-4
L-**Isoleucine**	5 .times. 10.sup.-4
L-**Leucine**	1 .times. 10.sup.-3
L-Lysine.HCl	1 .times. 10.sup.-3
L-Methionine	2 .times. 10.sup.-4
	1 .times. 10.sup.-4
L-**Phenylalanine**	2 .times. 10.sup.-4
L-Proline	1 .times. 10.sup.-4
L-Serine	3 .times. 10.sup.-4
L-Threonine	3 .times. 10.sup.-4
	1 .times. 10.sup.-4
L-Tryptophan	2 .times. 10.sup.-5
L-Tyrosine	1 .times. 10.sup.-4
L-**Valine**	1 .times. 10.sup.-3
VITAMINS	
d-Biotin	3 .times. 10.sup.-8
Folinic Acid (Ca salt).5H.sub.2 O	
	1 .times. 10.sup.-6
DL- **alpha-Lipoic Acid**	
	1 .times. 10.sup.-8
Niacinamide	5 .times. 10.sup.-5
D-Pantothenic Acid	
	1 .times. 10.sup.-4
	5 .times. 10.sup.-5
(Hemi-Ca salt)	
Pyridoxine.HCl	1 .times. 10.sup.-5
Riboflavin	1 .times. 10.sup.-8
Thiamin.HCl	1 .times. 10.sup.-5
Vitamin B12	1 .times. 10.sup.-8
OTHER ORGANIC	
COMPONENTS	
Adenine	1 .times. 10.sup.-6
Choline Chloride	1 .times. 10.sup.-4
D-Glucose	5.56 .times. 10.sup.-3
myo-**Inositol**	1 .times. 10.sup.-4
	4 .times. 10.sup.-5
Putrescine.2HCl	1 .times. 10.sup.-9
Sodium Pyruvate	1 .times. 10.sup.-3
Thymidine	1 .times. 10.sup.-7
BULK INORGANIC SALTS	
CaCl.sub.2.2H.sub.2 O	
	1.6. . .

DRWD FIG. 1 is a graph showing the growth response curve of HMSC for variation of the concentration of **arginine** in MCDB 131M+5% dFBS and 0.5% dBPE. Cell growth is assessed as described in Example 3 as total colony area (mm.sup.2)/dish. The concentration of **arginine** was measured in molar (moles/liter) units. Concentration

DRWD is plotted on a logarithmic scale. As shown in Table 1, the optimal concentration of **arginine** chosen for use in MCDB 120 was 1 .times.10.sup.-3 M.

DRWD . . . high concentrations. In FIG. 1, the HMSC growth response curve as a function of the variation of the concentration of **arginine** is shown. As noted in Table 1, the optimal concentration of **arginine** chosen for MCDB 120 was 1.times.10.sup.-3 M.

DRWD Optimization of MCDB 131M for growth of HMSC indicated that **arginine**, methionine, threonine, pantothenate, and **inositol** should be increased. In addition, although there were not well defined requirements for iron, zinc, or copper in media containing. . .

DRWD Individual growth-response titrations in MCDB 131M also suggested that reductions in the concentrations of **cysteine**, **glutamine**, tyrosine, lipoic acid, and phenol red might be beneficial. However, when all of these reductions (except phenol red) were combined. . .

DRWD In summary, MCDB 120 differs from MCDB 131M in its levels of

arginine, methionine, threonine, pantothenate, **inositol**
, iron, zinc, and copper, all of which have been increased, and in its level of phenol red, which has been. . .

DRWD . . . Sci. USA 81:5435-5439; Tsao, M. C. eta! . (1982) J. Cell. Physiol. 110:219-229), at a concentration of 0.5% (70 .mu.g/ml **protein**) supported growth equivalent to that obtained with 0.5% embryo extract, and that dialyzed BPE (dBPE) was equally effective. It was. . .

DRWD . . . most of the bovine serum albumin removed. This preparation is a
relatively crude ammonium sulfate fraction of fetal bovine serum **protein**, and cannot be considered to be fully "defined" in the strictest sense of the term. Hence, media containing it are. . .

DRWD . . . growth media makes it necessary to ask whether the cells may have lost their ability to fuse and express muscle-specific **proteins**. Evidence that this is not the case has been obtained by growing cultures to confluency in the growth media and. . .

DRWD . . . to DMEI, the HMSC cells undergo extensive fusion to form multinucleate myotubes. This is accompanied by a major increase in **creatine** kinase specific activity. Preliminary electrophoretic studies indicate that most of the increase is due to enhanced levels of the MM and MB isozymes of **creatine** kinase. Spontaneous twitching has also been observed in HMSC cultures allowed to remain in DMEI for six days after transfer. . . growth media. Control cultures that are transferred into fresh DS or SF media show very little fusion or increase in **creatine** kinase specific activity.

DRWD . . . of that carried over from the growth medium is not strictly needed for differentiation of HMSC, although the level of **creatine** kinase obtained in unsupplemented DME remains somewhat less than in DME plus insulin. The extent of differentiation in DME plus. . .

DRWD . . . are only slightly less supportive of differentiation than DME. Cultures transferred into these media plus insulin exhibit substantial fusion and **creatine** kinase synthesis, but not as extensive as in DMEI. Intermediate levels of differentiation are also observed in F10
or F12 plus insulin. Collagen coating of the dishes somewhat increases the level of **creatine** kinase synthesis in most media, but is not essential for differentiation of HMSC.

DRWD . . . R. G. et al. (1987) supra; Hammond, S. L. et al. (1984) supra).
Full strength BPE contains approximately 14 mg/ml **protein** (determined by the Lowry method with serum albumin as a standard). Dialyzed BPE (dBPE) was prepared by dialysis against deionized. . .

DRWD . . . were included in all routine clonal growth assays. Quantitative
data on extent of differentiation were obtained by spectrophotometric determination of **creatine** kinase specific activity in dense cultures. For the 10 **creatine** kinase assays, a cell suspension was prepared with trypsin-EDTA as described above, counted, and diluted to 12,500-24,000 cells per ml. . .

DRWD . . . thawed, scraped from the culture surface, and vortexed, still in the glycylglycine buffer. Commercial kits were used to determine total **creatine** kinase activity (Sigma) and total **protein** (Biorad, Richmond, Calif.), and **creatine** kinase specific activity was calculated from the two values. In addition, **creatine** kinase isozyme distributions of selected cell lysates were examined with a commercial kit (Tital Gel-PC CPK-isozyme kit, Helena Laboratories, Beaumont,. . .

DRWD . . . that use of various chromatographic methods, including reversed-phase HPLC, had failed to separate any active contaminant from the bulk fetuin **protein**. This suggested that the mitogenic activity for muscle cell might reside in fetuin itself.

DRWD . . . Biophys. Res. Comm. 80:1013-1021) that exploits the solubility of fetuin in polyethylene glycol solutions to separate it from other serum **proteins** (Loskutoff, D. J. (1978) J. Cell. Physiol.

96:361-370) was used. This method proved to be effective, and after separation from. . .

DRWD . . . AS and the supernatant 50 AS SN. 50 AS was redissolved and precipitated with 25% PEG, which precipitates virtually all **proteins** except fetuin (Loskutoff, D. J. (1978) J. Cell. Physiol., 96:361-370). The 25% PEG precipitate was redissolved, and loaded onto a. . .

DRWD . . . was carried out according to the method of Laemmli (21) in 7.5% polyacrylamide gel using a vertical slab gel apparatus (**Protein** II Slab Cell, BioRad). Fractions from FPLC anion-exchange chromatography were desalted and concentrated by ultrafiltration using Centricoh-3 membrane (Amicon) before. . .

DRWD . . . the biological activity was eluted from the column in fractions 14, 15 and 16, just after the bulk of the **protein**. Fractions 14, 15 and 16 were pooled and used to obtain a dose-response curve for stimulation of growth of HMSC. . .

DRWD . . . and indicates further that fetuin itself is not responsible for the growth. However, there were still a large number of **proteins** in the combined fractions, covering the whole range of resolution of the 7.5% polyacrylamide gel.

DRWD . . . K. (1985) In Vitro Cell. Der. Biol 21:636-640). However all of the fetuin-replacing activity is above 70 kDa and large **proteins** become more prominent as the activity is concentrated, suggesting that it might be a macroglobulin or a related **protein**.

DRWD . . . separated from fetuin. Thus, the biological activity of Pedersen fetuin appears to be due to the presence of other serum **proteins** that are not completely removed from this relatively crude serum fraction. A 99-fold enrichment of the mitogenic activity for HMSC with 30% recovery has been achieved. However, the most active fractions still contain numerous **protein** bands and additional purification will be needed for precise characterization of the active substance (s) in these fractions.

AN 94:55475 USPATFULL
TI Media for normal human muscle satellite cells
IN Ham, Richard G., Boulder, CO, United States
St. Clair, Judith A., Boulder, CO, United States
Nie, Zetan, Boston, MA, United States
PA University of Colorado Foundation, Inc., Boulder, CO, United States (U.S. corporation)
PI US 5324656 19940628 <--
AI US 1992-928958 19920812 (7)
DCD 20090901
RLI Division of Ser. No. US 1988-265785, filed on 1 Nov 1988, now patented, Pat. No. US 5143842
DT Utility
EXNAM Primary Examiner: Elliott, George C.
LREP Greenlee & Winner
CLMN Number of Claims: 15
ECL Exemplary Claim: 8
DRWN 5 Drawing Figure(s); 4 Drawing Page(s)
LN.CNT 1409
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 70 OF 82 USPATFULL
PI US 5318987 19940607 <--
SUMM . . . 1520), and the likewise lipid-soluble, but unstable, temperature- and light-sensitive vitamin E (ibid, No. 9832, page 1437) and the lipid-insoluble **ascorbic acid** (ibid, No. 846 page 120) are used as preservatives.
SUMM A.sub.5 --an **ascorbic acid** (derivative) radical

##STR4## in which

SUMM . . . radicals of the type --O--, --S-- and/or --NR.sup.10 -- are separated from one another by at least 1 carbon or **phosphorus** atom;

SUMM where only 1 or 2 radicals R.sup.5 -R.sup.8 contain Q or are identical to Q (=an **ascorbic acid** radical).

SUMM . . . piperazine, mono-, di- and triethanolamine, ethyldiethanolamine, N-butylethanolamine, tris(hydroxymethyl)aminomethane and the like. Suitable amine salts are, for example, those of tryptamine, **cysteine** and the basic amine salts of lyeins and **arginine**. Suitable quaternary ammonium cations are, for example, tetramethylammonium and benzyltrimethylammonium. These cations can also be used for salt formation of. . .

DETD 29) 2-O-Octoadecyl-3-O-(3,5-di-tert.-butyl-4-hydroxyphenylmethyl) **ascorbic acid** ##STR45##

DETD 33) 2-O-(2-Cholesteryloxyethyl)**ascorbic acid** ##STR49##

DETD 34) 6-O-Octadecanoyl-2-O-(O*,O*-diethylphosphoryl)-**ascorbic acid** ##STR50##

DETD 35) 5-O,6-O-Dioctadecanoyl-2-O-(O*,O*-diethylphosphoryl)-**ascorbic acid** ##STR51##

DETD 52) N-Octadecyl-DL-.**alpha**.-**lipoic acid** amide ##STR68##

DETD a) 100 mg (0.48 mmol) of DL-.**alpha**.-**lipoic acid** were dissolved in 2 ml of 0.25N aqueous sodium hydrogen carbonate solution and 20 mg of sodium borohydride were added.. . .

DETD . . . of BHT (=butylated hydroxytoluene) or 2,6-di-tert.-butyl-4-(7-nonynoyl)-phenol (=compound according to Example 17] or ethyl 2-(3,5-di-tert.-butyl-4-hydroxybenzyl)-3-oxo-docosanoate (=compound according to Example 18) or N-octadecyl-DL-.**alpha**.-**lipoic acid** amide (=compound according to Example 52) was added and the mixture was used in the customary manner as frying fat.. . . probably comes about as a result of their lipophilic side chains and, therefore, improved lipophilic interaction. The advantageous action of N-octadecyl-DL-.**alpha**.-**lipoic acid** amide is particularly surprising, although this preparation has no recognizable antioxidative component.

DETD . . . <0.1 >50.0

Cpd. acc. to Ex. 27	>10.0	>1.0
Cpd. acc. to Ex. 28	<0.1	>50.0
Cpd. acc. to Ex. 29		
0.711	>1.0	>10.0
Ascorbic acid analog		
Ascorbic acid.sup.(1)		
2.99	>10.0	<0.1
Ascorbyl palmitate.sup.(1)		
	<0.1	>0.1
Cpd. acc. to Ex. 34		
0.052	<0.1	>1.0
Cpd. acc. to Ex. 35		
0.053	<0.1	>1.0

Cpd.. . .

CLM What is claimed is:

. . . 2 radicals selected from --O--, --S-- and --NR.sup.10 -- are separated from one another by at least 1 carbon or **phosphorus** atom; and X is a lipophilic component X.sub.1, a cholane derivative radical, of the following formula ##STR114## in which R.sup.13. . .

AN 94:49168 USPATFULL|

TI Lipid-selective antioxidants and their preparation and use|

IN Weithmann, Klaus-Ulrich, Hofheim am Taunus, Germany, Federal Republic of

Wess, Gunther, Erlensee, Germany, Federal Republic of

Seiffge, Dirk, Mainz, Germany, Federal Republic of

PA Hoechst Aktiengesellschaft, Frankfurt am Main, Germany, Federal Republic
of (non-U.S. corporation)
PI US 5318987 19940607 <--
AI US 1991-638321 19910107 (7)
PRAI DE 1990-4000397 19900109
DT Utility|
EXNAM Primary Examiner: Ivy, C. Warren; Assistant Examiner: Owens, A. A.|
LREP Finnegan, Henderson, Farabow, Garrett & Dunner|
CLMN Number of Claims: 3|
ECL Exemplary Claim: 1|
DRWN No Drawings
LN.CNT 1039|
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 71 OF 82 USPATFULL
PI US 5286622 19940215 <--
SUMM . . . benzotriazoles), mercaptopyrimidines, mercaptotriadines, a thioketo compound, azaindenes (for example, triazaindenes, tetraazaindenes, and pentaazaindenes), benzenesulfonic acids, benzenesulfinic acids, benzenesulfonic amides, and .**alpha**.-**lipoic acid** can be advantageously used as these compounds. Representative examples thereof include 1-phenyl-2-mercaptotetrazole, 4-hydroxy-6-methyl-1,3,3a,7-tetrazaindene, 2-mercaptobenzothiazole, and 5-carboxybutyl-1,2-dithiolane.
SUMM . . . the light-sensitive element of the present invention but hydrophilic binders other than gelatin can be used as well. For example,
proteins (gelatin derivatives, graft polymers of gelatin with other polymers, albumin, and casein), cellulose derivatives (hydroxyethyl cellulose, carboxymethyl cellulose, and cellulose. . .

SUMM Phenidones, p-aminophenols and **ascorbic acid** are preferably used as a developing aid in combination with the above-described developing agents. Phenidones are preferably used in combination.
AN 94:13421 USPATFULL
TI Light-sensitive element for silver salt diffusion transfer method
IN Waki, Koukichi, Kanagawa, Japan
PA Fuji Photo Film Co., Ltd., Kanagawa, Japan (non-U.S. corporation)
PI US 5286622 19940215 <--
AI US 1993-54981 19930430 (8)
PRAI JP 1992-111637 19920430
DT Utility
EXNAM Primary Examiner: Schilling, Richard L.
LREP Sughrue, Mion, Zinn, Macpeak & Seas
CLMN Number of Claims: 6
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 1055
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 72 OF 82 USPATFULL
PI US 5143842 19920901 <--
SUMM . . . combination with DME, include M199 (Askanas, V. and Engel, W.K.
(1975) Neurology 25:58-67; Konigsberg, I.R. (1963) supra), MEM plus nonessential **amino acids**, pyruvate and additional vitamins (Miranda, A.F. et al. (1979) in Muscle regeneration, Mauro, S. et al. (eds.), Raven Press, New. . .
SUMM . . . the composition of MCDB 120. Clonal growth of HMSC was sensitive in particular to the concentrations of the medium components L-**arginine**, L-methionine, L-threonine, D-pathothenic acid and myo-**inositol**. It is believed that MCDB 120 is the first optimized basal nutrient medium that has been developed specifically for

the. . .

SUMM

TABLE 1

Composition of MCDB 120 and MCDB 131M

	MCDB 120 moles/liter	MCDB 131M.sup.1 moles/liter
AMINO ACIDS		
L-**Alanine**	3 .times. 10.sup.-5	
L-**Arginine**.HCl	1 .times. 10.sup.-3	
		3 .times. 10.sup.-4
L-Asparagine.H.sub.2 O		
	1 .times. 10.sup.-4	
L-Aspartic Acid	1 .times. 10.sup.-4	
L-**Cysteine**.HCl.H.sub.2 O		
	2 .times. 10.sup.-4	
L-Glutamic Acid	3 .times. 10.sup.-5	
L-**Glutamine**	1 .times. 10.sup.-2	
Glycine	3 .times. 10.sup.-5	
L-Histidine.HCl.H.sub.2 O		
	2 .times. 10.sup.-4	
L-**Isoleucine**	5 .times. 10.sup.-4	
L-**Leucine**	1 .times. 10.sup.-3	
L-Lysine.HCl	1 .times. 10.sup.-3	
L-Methionine	2 .times. 10.sup.-4	
		1 .times. 10.sup.-4
L-**Phenylalanine**	2 .times. 10.sup.-4	
L-Proline	1 .times. 10.sup.-4	
L-Serine	3 .times. 10.sup.-4	
L-Threonine	3 .times. 10.sup.-4	
		1 .times. 10.sup.-4
L-Tryptophan	2 .times. 10.sup.-5	
L-Tyrosine	1 .times. 10.sup.-4	
L-**Valine**	1 .times. 10.sup.-3	
VITAMINS		
d-Biotin	3 .times. 10.sup.-8	
Folinic Acid (Ca salt).5H.sub.2 O		
	1 .times. 10.sup.-6	
DL- **alpha-Lipoic Acid**		
	1 .times. 10.sup.-8	
Niacinamide	5 .times. 10.sup.-5	
D-Pantothenic Acid		
	1 .times. 10.sup.-4	
		5 .times. 10.sup.-5
(Hemi-Ca salt)		
Pyridoxine.HCl	1 .times. 10.sup.-5	
Riboflavin	1 .times. 10.sup.-8	
Thiamin.HCl	1 .times. 10.sup.-5	
Vitamin B12	1 .times. 10.sup.-8	
OTHER ORGANIC COMPONENTS		
Adenine	1 .times. 10.sup.-6	
Choline Chloride	1 .times. 10.sup.-4	
D-Glucose	5.56 .times. 10.sup.-3	
myo-**Inositol**	1 .times. 10.sup.-4	
		4 .times. 10.sup.-5
Putrescine.2HCl	1 .times. 10.sup.-9	
Sodium Pyruvate	1 .times. 10.sup.-3	
Thymidine	1 .times. 10.sup.-7	
BULK INORGANIC SALTS		
CaCl.sub.2.2H.sub.2 O		
	1.6. . .	

DRWD FIG. 1 is a graph showing the growth response curve of HMSC for variation of the concentration of **arginine** in MCDB 131M +5% dFBS and 0.5% dBPE. Cell growth is assessed as described in Example 3 as

total colony area (mm.sup.2)/dish. The concentration of **arginine** was measured in molar (moles/liter) units. Concentration is plotted on a

logarithmic scale. As shown in Table 1, the optimal concentration of **arginine** chosen for use in MCDB 120 was 1 .times. 10.sup.-3 M.

DETD . . . high concentrations. In FIG. 1, the HMSC growth response curve as a function of the variation of the concentration of **arginine** is shown. As noted in Table 1, the optimal concentration of **arginine** chosen for MCDB 120 was 1 .times. 10.sup.-3 M.

DETD Optimization of MCDB 131M for growth of HMSC indicated that **arginine**, methionine, threonine, pantothenate, and **inositol** should be increased. In addition, although there were not well defined requirements for iron, zinc, or copper in media containing. . .

DETD Individual growth-response titrations in MCDB 131M also suggested that reductions in the concentrations of **cysteine**, **glutamine**, tyrosine, lipoic acid, and phenol red might be beneficial. However, when all of these reductions (except phenol red) were combined. . .

DETD In summary, MCDB 120 differs from MCDB 131M in its levels of **arginine**, methionine, threonine, pantothenate, **inositol**, iron, zinc, and copper, all of which have been increased, and in its level of phenol red, which has been. . .

DETD . . . Acad. SCi. USA 81:5435-5439; Tsao, M.C. et al. (1982) J. Cell. Physiol. 110:219-229), at a concentration of 0.5% (70 .mu.g/ml **protein**) supported growth equivalent to that obtained with 0.5% embryo extract, and that dialyzed BPE (dBPE) was equally effective. It was. . .

DETD . . . O. (1947) J. Phys. Colloid Chem. 51:164-171). This preparation is a relatively crude ammonium sulfate fraction of fetal bovine serum **protein**, and cannot be considered to be fully "defined" in the strictest sense of the term. Hence, media containing it are. . .

DETD . . . growth media makes it necessary to ask whether the cells may have lost their ability to fuse and express muscle-specific **proteins**. Evidence that this is not the case has been obtained by growing cultures to confluency in the growth media and. . .

DETD . . . to DMEI, the HMSC cells undergo extensive fusion to form multinucleate myotubes. This is accompanied by a major increase in **creatine** kinase specific activity. Preliminary electrophoretic studies indicate that most of the increase is due to enhanced levels of the MM and MB isozymes of **creatine** kinase. Spontaneous twitching has also been observed in HMSC cultures allowed to remain in DMEI for six days after transfer. . . growth media. Control cultures that are transferred into fresh DS or SF media show very little fusion or increase in **creatine** kinase specific activity.

DETD . . . of that carried over from the growth medium is not strictly needed for differentiation of HMSC, although the level of **creatine** kinase obtained in unsupplemented DME remains somewhat less than in DME plus insulin The extent of differentiation in DME plus.

. .

DETD . . . are only slightly less supportive of differentiation than DME. Cultures transferred into these media plus insulin exhibit substantial fusion and **creatine** kinase synthesis, but not as extensive as in DMEI. Intermediate levels of differentiation are also observed in F10

or F12 plus insulin. Collagen coating of the dishes somewhat increases the level of **creatine** kinase synthesis in most media, but is not essential for differentiation of HMSC.

DETD . . . described (Ham, R.G. et al. (1987) supra: Hammond, S.L. et al. (1984) supra). Full strength BPE contains approximately 14 mg/ml **protein** (determined by the Lowry method with serum albumin as a standard). Dialyzed BPE (dBPE) was prepared by dialysis against deionized. . .

DETD . . . were included in all routine clonal growth assays. Quantitative

data on extent of differentiation were obtained by spectrophotometric determination of **creatine** kinase specific activity in dense cultures. For the **creatine** kinase assays, a cell suspension was prepared with trypsinEDTA as described above, counted, and diluted to 12,500-24,000 cells per ml. . .

DETD . . . thawed, scraped from the culture surface, and vortexed, still in the glycylglycine buffer. Commercial kits were used to determine total **creatine** kinase activity (Sigma) and total **protein** (Biorad, Richmond, Calif.), and **creatine** kinase specific activity was calculated from the two values. In addition, **creatine** kinase isozyme distributions of selected cell lysates were examined with a commercial kit (Tital Gel-PC CPK-isozyme kit, Helena Laboratories, Beaumont,. . .

AN 92:72396 USPATFULL
TI Media for normal human muscle satellite cells
IN Ham, Richard G., Boulder, CO, United States
St. Clair, Judith A., Boulder, CO, United States
PA The University of Colorado Foundation, Inc., Boulder, CO, United States (U.S. corporation)
PI US 5143842 19920901 <--
AI US 1988-265785 19881101 (7)
DT Utility
EXNAM Primary Examiner: Doll, John; Assistant Examiner: Elliott, George C.
LREP Greenlee & Winner
CLMN Number of Claims: 22
ECL Exemplary Claim: 1,11
DRWN 3 Drawing Figure(s); 3 Drawing Page(s)
LN.CNT 961
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 73 OF 82 USPATFULL
PI US 5141741 19920825 <--
DETD . . . moisturizers, lower alcohols, thickeners, antioxidants, chelating agents, pH-controlling agents, antiseptics, perfumes, coloring agents, conventional UV absorbers, UV scattering agents, vitamins, **amino acids** and the like. Usable oleaginous materials include oils and fats such as olive oil, jojoba oil, castor oil, cacao butter,. . . glycerin, 1,3-butylene glycol, propylene glycol, sorbitol, polyethylene glycol, dipropylene glycol and the like, NMF (natural moisturizing factor) materials such as **amino acids**, sodium lactate, sodium pyrrolidone carboxylate and the like and water-soluble polymeric materials such as hyaluronic acid, collagen, mucopolysaccharides, chondroitin sulfate. . . materials such as carboxyvinyl polymers, polyvinyl alcohol and the like. Usable antioxidants include dibutyl hydroxytoluene, butyl hydroxy anisole, propyl gallate, **ascorbic acid** and the like. Usable chelating agents include disodium ethylenediamine tetraacetate, ethane hydroxy diphosphate, pyrophosphates, hexametaphosphates, citric acid, tartaric acid, gluconic. . . vitamin B, vitamin C, vitamin D, vitamin E, vitamin F, vitamin K, vitamin P, vitamin U, carnitine, ferulic acid, .gamma.-oryzanol, .**alpha**.-**lipoic acid**, orotic acid and derivatives thereof and the like. Usable **amino acids** include glycine, **alanine**, **valine**, **leucine**, **isoleucine**, serine, threonine, **phenylalanine**, tyrosine, tryptophan, cystine, methionine, proline, hydroxyproline, **arginine**, hystidine and lysine as well as derivatives thereof. Although the skin-care preparations compounded with the ellagic acid compound exhibit a. . .

AN 92:70130 USPATFULL
TI Anti-sunburn skin-care preparation
IN Ishida, Keiichiro, Tokyo, Japan
Sato, Yoshimi, Tokyo, Japan
Egawa, Makoto, Inzai, Japan
Takeuchi, Keiji, Tokyo, Japan

PA Lion Corporation, Tokyo, Japan (non-U.S. corporation)
PI US 5141741 19920825 <--
AI US 1989-444960 19891204 (7)
PRAI JP 1988-311401 19881209
DT Utility
EXNAM Primary Examiner: Ore, Dale R.
LREP Hopgood, Calimafde, Kalil, Blaustein & Judlowe
CLMN Number of Claims: 9
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 512
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 74 OF 82 USPATFULL
PI US 5126327 19920630 <--
AB A melanocyte-stimulating hormone inhibitor has an **amino acid** sequence represented by the following formula [I], [II] or [III] in the molecule:
AB wherein His, Ser, Arg, Trp, Leu, Ala and Cys represent L-- or D-histidine, serine, **arginine**, tryptophan, **leucine**, **alanine** and **cysteine** residues, respectively. The melanocyte-stimulating hormone inhibitor and an external preparation to be applied to the skin which contains the inhibitor. . .
SUMM The present invention provides a melanocyte-stimulating hormone inhibitor having an **amino acid** sequence represented by the following formula [I], [II], or [III] in the molecule:
SUMM wherein His, Ser, Arg, Trp, Leu, Ala and Cys represent L- or D-histidine, serine, **arginine**, tryptophan, **leucine**, **alanine** and **cysteine** residues, respectively.
DETD Any compounds having an **amino acid** sequence represented by the above formula [I] or [II] in the molecule can be used in the present invention. Among. . .
DETD . . . Arg and Trp are preferably L-His, L-Arg and L-Trp residues, respectively, X and P each represent a hydrogen, acyl group, **amino acid** residue or acylated derivative thereof, peptide residue having 2 to 40, preferably 2 to 20 **amino acid** residues or acylated derivative thereof, X and P are preferably an acyl group, acylated **amino acid** residue or acylated peptide residue, the acyl group and acylated groups have 1 to 12, preferably 1 to 6 carbon atoms, Y and Q each represent a hydroxyl group, amino group, **amino acid** residue or amidated derivative thereof, peptide residue having 2 to 40, preferably 2 to 20 **amino acid** residues or amidated derivative thereof, and Y and Q are preferably an amino group, amidated **amino acid** residue or amidated peptide residue.
DETD Various combinations of the **amino acid** residues and peptide residues of X, Y, P and Q can be used so far as they do not inhibit the function of antagonizing MSH. The peptide residues are preferably physiologically inert. As for the stereostructure of each **amino acid** residue, it may be either L- or D-type. Those derived from artificial **amino acids** such as norleucine and norvaline are also usable. X, Y, P and Q may have a sugar chain.
DETD . . . the compound of the present invention to various proteases can be improved to exhibit the MSH-inhibition effect by employing a D-**amino acid** residue or synthetic **amino acid** residue as X, Y, P or Q.
DETD The **amino acid** sequence of X, Y, P or Q preferably contains -Ser-Tyr-Ser- in order to exhibit a high affinity for the ligand.. . . activity of the product can be improved by incorporating biotin or the like into the above-described sequence of the three **amino acids**.
DETD The molecular weight of the compound of the general formula [IV] [V]

comprising at least four **amino acid** residues as shown above is 584 to 10,000, preferably 600 to 6,000 and particularly preferably 600 to 3000. The number of the **amino acid** residues is 4 to 84, preferably 4 to 44 and particularly preferably 4 to 24.

DETD Any compounds having the **amino acid** sequence as shown by the formula [III] can be used in the present invention. Among them, peptides represented by the. . .

DETD wherein X.sub.1 represents a hydrogen, acyl group, **amino acid** residue, peptide residue having 2 to 40, preferably 2 to 20 **amino acid** residues or acylated derivative thereof, and X.sub.1 is preferably a hydrogen, acyl group, peptide residue having an **amino acid** sequence of -Ile-Leu or -Leu-His-Ala-Leu-Gln-Leu-Leu-Leu-Ile-Leu or acylated derivative thereof, the acyl group or acylated derivative has 1 to 12 carbon atoms, preferably 1 to 6 carbon atoms; and Y.sub.1 represents a hydroxyl group, amino group, **amino acid** residue, amidated derivative thereof, peptide residue having 2 to 36, preferably 2 to 16 peptide residues or amidated derivative thereof and Y.sub.1 is preferably a hydroxyl group, amino group, peptide residue having an **amino acid** sequence of Ile-Ser-Pro-Gly-Arg-Arg-or amidated derivative thereof.

DETD Various combinations of the **amino acid** residues and peptide residues of X.sub.1 and Y.sub.1 can be employed so far as they do not inhibit the MSH-inhibiting. . .

DETD The molecular weight of the compound of the formula [VI] comprising at least five **amino acid** residues as shown above is 532 to 10,000, preferably 532 to 6,000 and particularly preferably 532 to 4,000. The number of the **amino acid** residues is 5 to 81, preferably 5 to 41 and particularly preferably 5 to 31.

DETD In tables 1 and 2, the **amino acid** residues constituting the peptide are shown by abbreviations according to IUPAC, L-**amino acids** are shown without "L-" and D-**amino acids** are shown with "D" such as "D-Ser". Synthetic **amino acid** residues, i.e., norleucine and norvaline residues, are shown as Nle and Nva, respectively. In line with the ordinary mode of expression, the **amino acid** terminal (N-terminal) of the peptide is shown on the left and the carboxyl terminal (C-terminal) thereof is shown on the. . .

DETD TABLE 1 Compound of formula [IV] No. .rarw. X Sequence of essential **amino acids** Y .fwdarw. 1 H--His--Ser--Arg--Trp--OH 2 Ac--His--Ser--Arg--Trp--NH.sub.2 3 Ac--His--D--Ser--Arg--Trp--NH.sub.2 4 H--Glu--His--Ser--Arg--Trp--Gly--OH 5 Ac--Glu--His--Ser--Arg--Trp--Gly--NH.sub.2 6 Ac--Glu--His--D--Ser--Arg--Trp--Gly--NH.sub.2 7 H--Met--Glu--His--Ser--Arg--Trp--Gly--OH 8 Ac--Met--Glu--His--Ser--Arg--Trp--Gly--NH.sub.2 9 Ac--Met--Glu--His--D--Ser--Arg--Trp--Gly--NH.sub.2. . . -D--Ser--Arg--Trp--Gly--Lys--Pro--Val--NH.sub.2 61 H--Ser--Tyr--Phe--Met- -Glu--His--Ser--Arg--Trp--Gly--Lys--Pro--Val--OH 62 Ac--Ser--Tyr--Phe--Me t--Glu--His--Ser--Arg--Trp--Gly--Lys--Pro--Val--NH.sub.2 63 Ac--Ser--Tyr- -Phe--Met--Glu--His--D--Ser--Arg--Trp--Gly--Lys--Pro--Val--NH.sub.2 Compound of formula [V] No. .rarw. P Sequence of essential **amino acids** Q .fwdarw.

64

H--Trp--Arg--Ser--His--OH 65 Ac--Trp--Arg--Ser--His--NH.sub.2 66 Ac--Trp--Arg--D--Ser--His--NH.sub.2 67 H--Cys--Trp--Arg--Ser--His--Gln--OH 68 Ac--Cys--Trp--Arg--Ser--His--Gln--NH.sub.2 69 Ac--Cys--Trp--Arg--D-- Ser--His--Gln--NH.sub.2 70 H--Cys--Trp--Arg--Ser--

His--Gln--Pro--OH 71 Ac--Cys--Trp--Arg--Ser--His--Gln--Pro--NH.sub.2.

DETD TABLE 2 Compound of formula [VI] No. .rarw. X.sub.1 Sequence of essential **amino acids** Y.sub.1 .fwdarw.
101 Leu--Ala--Cys--Ala --Arg 102 Ac--Leu--Ala--Cys--Ala--Arg--NH.sub.2
103 Leu--Ala--Cys--Ala--A rg--Ile 104
Ac--Leu--Ala--Cys--Ala--Arg--Ile--
NH.sub.2 105 Leu--Ala--Cys- -Ala--Arg--Ile--Ser 106 Ac--Leu--Ala--Cys--Ala--Arg--Ile--Ser--NH.sub.2 107 Leu--Ala--Cys--Ala--Arg--Ile--Ser--Pro 108. . .

DETD In the liquid phase method, an amino group of a starting **amino acid** is protected with benzyloxycarbonyl group, t-butoxycarbonyl group or the like and a carboxyl group of the other starting **amino acid** or peptide is protected with a benzyl ester or the like and they are coupled together in the presence of. . . removed and the product is purified to obtain the compound of the present invention. In the solid phase method, an **amino acid** at the C-terminal is coupled with a crosslinked polystyrene resin and then t-butoxycarbonyl **amino acid** is coupled with the N-terminal one by one. After completion of the reaction, the product is removed from the resin,. . .

DETD . . . alcohol, humectant, thickening agent, antiseptic, antioxidant, chelating agent, pH adjustor, perfume, colorant, U.V. ray absorber, U.V. ray scattering agent, vitamins, **amino acids** and water.

DETD . . . polyhydric alcohols such as glycerol, propylene glycol, 1,3-butylene glycol, sorbitol, polyglycerol, polyethylene glycol and dipropylene glycol; NMF components such as **amino acids**, sodium lactate and sodium pyrrolidone carboxylate; and water-soluble high-molecular substances such as hyaluronic acid, collagen, mucopolysaccharides and chondroitin sulfate.

DETD The antioxidants include, for example, dibutylhydroxytoluene, butylhydroxyanisole, propyl gallate and **ascorbic acid**. The chelating agents include, for example, disodium edetate, ethylenediaminetetraacetates, pyrophosphates, hexametaphosphates, citric acid, tartaric acid and gluconic acid. The pH. . .

DETD . . . vitamin B, vitamin C, vitamin D, vitamin E, vitamin F, vitamin K, vitamin P, vitamin U, carnitine, ferulic acid .gamma.-oryzanol, **alpha.-lipoic acid**, orotic acid and derivatives of them.

DETD The **amino acids** include, for example, glycine, **alanine**, **valine**, **leucine**, **isoleucine**, serine, threonine, **phenylalanine**, tyrosine, tryptophan, cystine, **cysteine**, methionine, proline, hydroxyproline, aspartic acid, glutamic acid, **arginine**, histidine, lysine and derivatives of them.

DETD Since the MSH-inhibitors of the formulae [I] and [II] of the present invention have a structure similar to the **amino acid** sequence of the minimum structure in the molecule, it is supposed that the inhibitor has a strong affinity for the. . .

DETD One on the hand, the peptide of the formula [III] has the sequence of the five **amino acid** residues shown by the formula [III] in its molecule, which sequence is complementary to **amino acid** sequence having MSH activity and which sequence is considered to have a high structural affinity for MSH, so that the. .

DETD Val or Pro which was the **amino acid** at the C-terminal was coupled with a crosslinked polystyrene resin. Then **amino acids** having the amino group protected with t-butoxycarbonyl group were coupled with the N-terminal one by one. After all the **amino acids** had been coupled with the resin, the amino terminal was acetylated, the peptide having the protective group was removed from. . .

DETD TABLE 6

Component	Amount (%)
1 Compound No. 14 in Table 1	0.01
2 Glycerol	4.0
3 Carboxyvinyl polymer	0.5
4 Purified water	balance

Tocopherol acetate.

0.2	
6 Ethanol	10.0
7 Polyoxyethylene (40)	hydrogenated
	0.5
castor oil	
8 Methylparaben	0.1
9 Perfume	small amount

DETD Arg which was the **amino acid** at the C-terminal was coupled with a crosslinked polystyrene resin. Then **amino acids** having the amino group protected with t-butoxycarbonyl group were coupled with the N-terminal one by one. After all the **amino acids** had been coupled with the resin, the peptide with the protective group was removed from the resin, and the protective. . .

DETD TABLE 10

Component	Amount (%)
1 Compound No. 135 in Table 2	0.01
2 Glycerol	5.0
3 Carboxyvinyl polymer	0.5
4 Purified water	balance

Tocopherol acetate.

0.1	
6 Ethanol	10.0
7 Polyoxyethylene (40)	hydrogenated
	0.8
castor oil	
8 Methylparaben	0.1
9 Perfume	small amount

CLM What is claimed is:

1. A melanocyte-stimulating hormone inhibitor consisting essentially of an **amino acid** sequence represented by the following general formula (IV), (V) or (VI): X-His-Ser-Arg-Trp-Y (IV) P-Trp-Arg-Ser-His-Q (V) X.sub.1 -Leu-Ala-Cys-Ala-Arg-Y.sub.1 (VI) wherein His, Ser, Arg, Trp, Leu, Ala and Cys represent L- or D-histidine, serine, **arginine**, tryptophan, **leucine**, **alanine** and **cysteine** residues, respectively, X and P each represent a hydrogen, an acyl group having 1 to 12 carbon atoms, an **amino acid** residue, or acylated derivative thereof having 1 to 12 carbon atoms, peptide residue having 2 to 40 **amino acid** residues or acylated derivative thereof, and Y and Q each represent a hydroxyl group, an amino group, an **amino acid** residue, or amidated derivative thereof, or a peptide residue having 2 to 40 **amino acid** residues or amidated derivative thereof, and X.sub.1 represents a hydrogen, an acyl group having 1 to 12 carbon atoms, an **amino acid** residue, or acylated derivative thereof having 1 to 12 carbon atoms, or a peptide residue having 2 to 40 **amino acid** residues or acylated derivative thereof, and Y.sub.1 represents a hydroxyl group,

an amino group, an **amino acid** residue, or amidated derivative thereof, or a peptide residue having 2 to 36 **amino acid** residues or amidated derivative thereof and wherein the peptide of the formula (IV) or (V) has a molecular weight in. . . . . The inhibitor according to claim 1, wherein X and P each represent an

acyl group, an acylated derivative of an **amino acid** residue or an acylated derivative of a peptide residue having 2 to 40 **amino acid** residues, and Y and Q each represent an amino group, an amidated **amino acid** residue or an amidated derivative of a peptide residue having 2 to 40 **amino acid** residues.

. . . The inhibitor according to claim 1, wherein X.sub.1 represents a hydrogen, an acyl group, or a peptide residue having an **amino acid** sequence of -Ile-Leu- or -Leu-His-Ala-Leu-Gln-Leu-Leu-Leu-Ile-Leu or acylated derivative thereof, and Y.sub.1 represents a hydroxyl group, an amino group, or a peptide residue having an **amino acid** sequence of -Ile-Ser-Pro-Gly-Arg-Arg- or an amidated derivative thereof.

. . . wherein X and P each represent an acyl group having 1 to 6 carbon atoms, an acylated derivative of an **amino acid** residue having 1 to 6 carbon atoms or an acylated derivative of a peptide residue having 2 to 20 **amino acid** residues, and Y and Q each represent an amino group, an amidated **amino acid** residue or an amidated derivative of a peptide residue having 2 to 20 **amino acid** residues.

. . . wherein X.sub.1 represents a hydrogen, an acyl group having 1 to 6 carbon atoms, or a peptide residue having an **amino acid** sequence of -Ile-Leu- or -Leu-His-Ala-Leu-Gln-Leu-Leu-Leu-Ile-Leu or an acylated derivative thereof, and Y.sub.1 represents a hydroxyl group, an amino group, or a peptide residue having an **amino acid** sequence of -Ile-Ser-Pro-Gly-Arg-Arg-or amidated derivative thereof.

. . . -His-Ser-Arg-Trp- [I]
-Trp-Arg-Ser-His [II]
-Leu-Ala-Cys-Ala-Arg- [III]
wherein

His, Ser, Arg, Trp, Leu, Ala and Cys represent L- or D-histidine, serine, **arginine**, tryptophan, **leucine**, **alanine** and **cysteine** residues, respectively, and an inert carrier.

9. A topical composition to inhibit melanocyte-stimulating hormone comprising an effective amount of an **amino acid** sequence represented by the following general formula (IV), (V) or (VI) to inhibit melanocyte stimulating hormone: X-His-Ser-Arg-Trp-Y (IV) P-Trp-Arg-Ser-His-Q (V) X.sub.1 -Leu-Ala-Cys-Ala-Arg-Y (VI) wherein

His, Ser, Arg, Trp, Leu, Ala and Cys represent L- or D-histidine, serine, **arginine**, tryptophan, **leucine**, **alanine** and histidine, serine, **arginine**, tryptophan, **leucine**, **alanine** and **cysteine** residues, respectively, X and P each represent a hydrogen, an acyl group having 1 to 12 carbon atoms, an **amino acid** residue, or acylated derivative thereof having 1 to 12 carbon atoms, peptide residue

having 2 to 40 **amino acid** residues or acylated derivative thereof, and Y and Q each represent a hydroxyl group, an amino group, an **amino acid** residue, or amidated derivative thereof, and or a peptide residue having 2 to 40 **amino acid** residues or amidated derivative thereof, and X.sub.1 represents a hydrogen, an acyl group having 1 to 12 carbon

atoms, an **amino acid** residue, or acylated derivative thereof having 1 to 12 carbon atoms, or a peptide residue having 2 to 40 **amino acid** residues or acylated derivative thereof, and Y.sub.1, represents a hydroxyl group, an amino group, an **amino acid** residue, or amidated derivative thereof, or a peptide residue having 2 to 36 **amino acid** residue or amidated derivative thereof, wherein the peptide of the formula (IV) or (V) has a molecular weight in the. . .

10. The composition according to claims 8 which contains 0.0000000001 to 1 wt. % of the **protein**.

11. The composition according to claims 9 which contains 0.0000000001 to 1 wt. % of the **protein**.

AN 92:53278 USPATFULL|
TI Melanocyte-stimulating hormone inhibitor and external preparation containing the same|
IN Takeuchi, Takuji, Sendai, Japan
Sato, Chikara, Sendai, Japan
Oba, Kenkichi, Funabashi, Japan
Sugiyama, Keikichi, Kanagawa, Japan
PA Lion Corporation, Tokyo, Japan (non-U.S. corporation)
PI US 5126327 19920630 <--
AI US 1990-497191 19900322 (7)
PRAI JP 1989-71215 19890323
JP 1989-93643 19890413
DT Utility|
EXNAM Primary Examiner: Lee, Lester L.; Assistant Examiner: Davenport, A. M.|
LREP Burns, Doane, Swecker & Mathis|
CLMN Number of Claims: 25|
ECL Exemplary Claim: 1|
DRWN No Drawings
LN.CNT 1094|
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 75 OF 82 USPATFULL
PI US 5122445 19920616 <--
DETD . . . limitation is imposed upon the binder provided that it has film forming properties, and examples of such binders include gelatin, **proteins** such as casein, cellulose compounds such as carboxymethylcellulose, hydroxyethylcellulose, acetylcellulose, diacetylcellulose and triacetylcellulose, sugars such as dextran, agar, sodium alginate. . .
DETD . . . colloids can be used for this purpose. For example, gelatin derivatives, graft polymer so fo ther polymers with gelatin, and **proteins** such as albumin and casein for example; cellulose derivatives such as hydroxyethylcellulose, carboxymethylcellulose and cellulose sulfate esters for example, sodium. . .
DETD . . . alkylcarboxylates, alkylsulfonates, alkylbenzenesulfonates, alkylnaphthalenesulfonates, alkylsulfate esters, alkylphosphate esters, N-acyl-N-alkyltaurines,sulfosuccinate esters,sulfoalkylpolyoxyethylene alkylphenyl ethers and polyoxyethylene alkylphosphate esters; amphoteric surfactants, such as **amino acids**, aminoalkylsulfonic acids, aminoalkyl sulfate or phosphate esters, alkylbetaines and amine oxides, and cationic surfactants, such as alkylamine salts, aliphatic and. . .
DETD . . . latex (average particle
600 mg/m.sup.2
size 0.05 .mu.m)
1,2-Bis(vinylsulfonylacetamido)ethane
140 mg/m.sup.2

Sodium N-oleoyl-N-methyltaurine
40 mg/m.sup.2
Poly(sodium styrenesulfonate)
20 mg/m.sup.2
Formulation (12) Protective Layer 2
Gelatin 1.0 g/m.sup.2
Ascorbic acid 30 mg/m.sup.2
Hydroquinone 190 mg/m.sup.2
Ethyl acrylate latex (average particle
240 mg/m.sup.2
size 0.05 .mu.m)
Poly(sodium styrenesulfonate)
3 mg/m.sup.2
2,4-Dichloro-6-hydroxy-1,3,5-triazine,
12 mg/m.sup.2
sodium salt
Formulation (13) Protective. . .
DETD

Formulation (27) Protective Layer 1
Gelatin 1.0 g/m.sup.2
.alpha.-Lipoic acid 10 mg/m.sup.2
Sodium dodecylbenzenesulfonate
5 mg/m.sup.2
Compound 4 40 mg/m.sup.2
Compound 8 20 mg/m.sup.2
Poly(sodium styrenesulfonate)
10 mg/m.sup.2
1-Phenyl-5-mercaptotetrazole
5 mg/m.sup.2
Compound 9 20 mg/m.sup.2
Ethyl acrylate. . .
AN 92:48981 USPATFULL
TI Silver halide photographic materials
IN Ishigaki, Kunio, Kanagawa, Japan
PA Fuji Photo Film Co., Ltd., Kanagawa, Japan (non-U.S. corporation)
PI US 5122445 19920616 <--
AI US 1990-540066 19900619 (7)
PRAI JP 1989-157142 19890620
JP 1989-295620 19891114
DT Utility
EXNAM Primary Examiner: McCamish, Marion E.; Assistant Examiner: Dote, Janis L.
LREP Sughrue, Mion, Zinn, Macpeak & Seas
CLMN Number of Claims: 20
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 1745
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 76 OF 82 USPATFULL
PI US 5089269 19920218 <--
DRWD FIG. 3 is a graph showing the stability with a lapse of time of the L-**ascorbic acid** of Example 7-1 and Comparative Example 7-1;
DRWD FIG. 6 is a graph showing the leakage characteristics of a cream base with a lapse of time of the L-**ascorbic acid** of Example 7-3 and Comparative Example 7-3.
DETD . . . beeswax, candelilla wax, whale wax, carnauba wax; vitamins such as vitamin A, B.sub.2, D, E; vitamin-like acting substances such as .**alpha.-lipoic acid**, ferulic acid; sunscreening agents such as ethyl paraaminobenzoate, oxybenzone, etc.; and various natural and synthetic flavors. These however, are not. .
.
DETD . . . invention, any method generally employed may be used, including, for example, the simple emulsification method by high

shearing force, the **amino acid** gel emulsification method utilizing the salt-out action of **amino acid** relative to the activating agent, and the emulsification method utilizing the gelling ability in oil of organophilic clay mineral.

DETD . . . any of the methods generally employed may be used, for example, a simple emulsification under a high shear pressure, an **amino acid** gel emulsification method utilizing the salt-out action of **amino acid**, and a clay emulsification method utilizing the gelling ability of organophilic clay mineral.

DETD . . . the water-soluble skin effective component to be formulated in the aqueous phase portion, there may be included vitamins, hormones, and **amino acids**, and specific examples of these further include vitamins such as thiamine hydrochloride, pyridoxin hydrochloride, nicotinic acid amide, calcium pantothenate, biotin, **inositol**, **ascorbic acid**, sodium ascorbate, rutin; hormones such as estradiol, cortizone, prednizolone; and **alanine**, glycine, lysine, pyrrolidone carboxylic acid, but these are not limited in the present invention, and any water-soluble starting materials which. . .

DETD . . . acid, hydrobromic acid, and organic electrolytes may include organic acids and water-soluble metal salts or ammonium salts of organic acids, **amino acids** and water-soluble metal salts, quaternary ammonium salts of **amino acids**, etc., which can be employed singly or as a mixture of two or more thereof. As the organic compound, methanol,. . .

DETD

Oil Phase		
Squalane	25.0	(part)
Ceresine	3.0	
Microcrystalline wax	1.5	
Lanolin	0.5	
Petrolatum	6.0	
Flavor	q.s.	
Preservative	q.s.	
Amino Acid W/O Gel		
Glycerine monooleate	4.0	
Monosodium L-glutamate	3.2	
Purified Water	12.8	
Aqueous Phase		
Oil components enclosing micro-capsules	7.0	
Propylene glycol	5.0	
Purified water	balance	

DETD . . . monosodium L-glutamate to be dissolved therein, and the solution was added to glycerine monooleate at 70.degree. C. to prepare an **amino acid** W/O gel by a homomixer. Then, the oil phase portion and the aqueous phase portion dissolved by heating at 70.degree.. . .

DETD . . . 5.0

Organic modified clay mineral obtained by treating 0.5 g of bentone-38 with 0.1 g of dimyristoyl lecithin	0.6
Microcrystalline wax	2.0
Butyl parahydroxybenzoate	0.1
Aqueous Phase	
L-ascorbic acid	2.0
Dipropylene glycol	5.0

Purified water	balance

DETD . . . lotion having the same amounts of squalane and vitamin A palmitate as the enclosed amounts in the oil phase and L-**ascorbic acid** in the aqueous phase without formulation of microcapsules in the above recipe (Comparative Example 7-1) was prepared, and a comparative. . . with the above Example was conducted of the stability with a lapse of time of the vitamin A palmitate and L-**ascorbic acid**.

DETD . . . vessels containing the respective emollient lotions in a thermostat at 50.degree. C., and the amounts of vitamin A palmitate and L-**ascorbic acid** remaining after 7, 14, 30, and 60 days were determined.

DETD . . . preparation of the emollient lotion is represented as 100%. Example 7-1 shows a slower reduction of vitamin A palmitate and L-**ascorbic acid** with a lapse of time, and thus shows an excellent in stability.

DETD

Oil Phase	
Squalane	25.0 (part)
Ceresine	3.0
Microcrystalline wax	1.5
Lanolin	0.5
Petrolatum	6.0
.gamma.-linoleinic acid	0.5
Amino Acid W/O Gel	
Glycerine monooleate	4.0
Monosodium L-glutamate	3.2
Purified water	12.8
Aqueous Phase	
Vitamin B.sub.6 hydrochloride	0.4
Propylene glycol	5.0
Purified water	balance

DETD . . . monosodium L-glutamate to be dissolved therein, and the solution was added to glycerine monooleate of 70.degree. C. to prepare an **amino acid** W/O gel by a homomixer, and then the oil phase portion and the aqueous phase portion dissolved by heating to. . .

DETD . . . was left to stand at room temperature for 10, 30, 60, 120, 180, and 360 days, and the amount of L-**ascorbic acid** migrated (leaked out) to the outer phase (cream base) of microcapsules was determined. The experiment method was conducted by separating. . and the microcapsule layer by centrifugation at 5000 rpm (with the microcapsule being the upper layer), and quantitatively determining the L-**ascorbic acid** contained in the cream base. As a Comparative Example, the same investigations were made for the microcapsules of nylon film enclosing L-**ascorbic acid** (Comparative Example 7-3). The microcapsules of nylon film were prepared by an interfacial polymerization of ethylenediamine and terephthalic acid chloride,. . .

DETD . . . are shown in FIG. 6, from which it can be seen that Example has a low level of leakage of L-**ascorbic acid** from the microcapsule for a long term, compared with the Comparative Example.

DETD . . . mixed oil of squalane : vitamin C palmitate=9:1 (Oil B), 10 g of oleic acid (Oil C), 10 g of **tocopherol** acetate (Oil D), 10 g of a mixed oil of neopentyl glycol didecanoate : ethylhexyl paradimethylaminobenzoate=7:3 (Oil E), 10 g. . .

CLM What is claimed is:
. . . consisting of animal and vegetable oils, hydrocarbon oils, ester oils, silicone oils, higher fatty acids, higher alcohols, sunscreening agents, vitamins, **alpha lipoic acid**, ferulic acid, and flavors and said solid or semi-solid oil component is at least one member selected from the group. . .
. . . consisting of animal and vegetable oils, hydrocarbon oils, ester oils, silicone oils, higher fatty acids, higher alcohols, sunscreening agents, vitamins, **alpha lipoic acid**, ferulic acid, and flavors and said solid or semi-solid oil component is at least one member selected from the group. . .
AN 92:12729 USPATFULL|
TI Cosmetic containing fine soft microcapsules|
IN Noda, Akira, Yokohama, Japan
Yamaguchi, Michihiro, Yokohama, Japan
Aizawa, Masanori, Yokohama, Japan
Kumano, Yoshimaru, Yokohama, Japan
PA Shiseido Company Ltd., Tokyo, Japan (non-U.S. corporation)
PI US 5089269 19920218 <--
AI US 1988-199977 19880527 (7)
PRAI JP 1987-281825 19871107
JP 1988-65318 19880318
JP 1988-93945 19880415
JP 1988-93947 19880415
JP 1988-95315 19880418
JP 1988-95316 19880418
JP 1988-95317 19880418
DT Utility|
EXNAM Primary Examiner: Page, Thurman K.; Assistant Examiner: Hulina, Amy L.|
LREP Sprung Horn Kramer & Woods|
CLMN Number of Claims: 2|
ECL Exemplary Claim: 1|
DRWN 6 Drawing Figure(s); 6 Drawing Page(s)|
LN.CNT 3057|
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 77 OF 82 USPATFULL
PI US 5084481 19920128 <--
SUMM . . . test in mouse and in the Randall Selitto inflammation pain test in rat. The effect is superior to that of **alpha-lipoic acid** by a factor of at least 1.9 and 2.8 respectively (peroral application).
SUMM . . . displays a good anti-inflammatory activity for example in carragheen-induced edema in the rat which is also superior to that of **alpha-lipoic acid** by a factor of at least 1.9 (peroral application). Dihydrolipoic acid also displays good inhibition of vasopermeability for example in. . .
SUMM The direction of the effect of dihydrolipoic acid is comparable to the effect of the known active substances **alpha-lipoic acid** and S-adenosyl-L-methionine, although the following differences exist in particular:
SUMM 2. The effect of dihydrolipoic acid is for example stronger than that of **alpha-lipoic acid** by a factor of 2-3.
SUMM Antioxidants which may for example be used are sodium sulfite, sodium hydrogen sulfite, sodium metabisulfite, **ascorbic acid**, ascorbyl palmitate, -myristate, -stearate, gallic acid, gallic acid alkyl ester, butylhydroxyanisol, nordihydroguaiacic acid, **tocopherols** as well as synergists (substances which bind heavy metals through complex formation, for example lecithin, **ascorbic acid**, phosphoric acid, ethylenediaminotetraacetic acid, citrate, tartrate). The addition of synergists substantially increases the antioxygenic effect of the antioxidants. Preservatives which. . .
SUMM . . . cations which are physiologically acceptable in the salt form. Examples are: acceptable alkaline or alkaline earth metals, ammonium

hydroxide, basic **amino acids** such as **arginine** and lysine, amines of the formula NR.sub.1 R.sub.2 R.sub.3 wherein the radicals R.sub.1, R.sub.2 and R.sub.3 are the same or. . . cyclic amino compounds having from 4 to 6 carbon atoms in the ring, such as piperidine, piperazine, pyrrolidone, morpholine; N-methylglucamine, **creatine**, tromethamine.

DETD . . . pale brown to brown, waxy mass which melts on heating to 55.degree. C. to a clear brown fluid and contains **-tocopherol**, ascorbylpalmitate, citric acid and lecithin. *

AN 92:7370 USPATFULL

TI Method of treating inflammatory diseases with pharmaceutical composition containing dihydrolipoic acid as active substance

IN Ulrich, Heinz, Niedernberg, Germany, Federal Republic of
Weischer, Carl-Heinrich, Bonn, Germany, Federal Republic of
Engel, Jurgen, Alzenau, Germany, Federal Republic of
Hettche, Helmut, Dietzenbach, Germany, Federal Republic of

PA Asta Pharma Aktiengesellschaft, Germany, Federal Republic of (non-U.S. corporation)

PI US 5084481 19920128 <--

AI US 1990-476042 19900208 (7)

PRAI DE 1989-3903758 19890209

DT Utility

EXNAM Primary Examiner: Waddell, Frederick E.; Assistant Examiner: Jordan, Kimberly R.

LREP Cushman, Darby & Cushman

CLMN Number of Claims: 2

ECL Exemplary Claim: 1

DRWN No Drawings

LN.CNT 532

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 78 OF 82 USPATFULL

PI US 5073545 19911217 <--

SUMM . . . consumers, and there have hitherto been used as effective components peroxides such as hydrogen peroxide, zinc peroxide and magnesium peroxide, **ascorbic acid**, glutathione, colloidal sulfur, and various natural substances. However, hydrogen peroxide and **ascorbic acid** have problems regarding stability, preservability, etc., and moreover it is hard to say that their effects are adequate. Further, glutathione. . .

DETD For example, in order to further disperse ellagic acid series compounds, basic **amino acids** such as **arginine** or monosaccharides such as glucose can be added in an amount of 0.001 to 30%, preferably 0.005 to 20% in. . . example, oils, water surfactants, humectants, lower alcohols, thickeners, antioxidants, chelating agents, pH-adjusting agents, antiseptics, perfume, coloring matters, ultraviolet absorbers, vitamins, **amino acids**, etc.

DETD . . . mentioned polyhydric alcohols such as glycerin, 1,3-butylene glycol, propylene glycol, sorbitol, polyethylene glycol and dipropylene glycol; NMF compounds such as **amino acids**, sodium lactate and sodium pyrrolidonecarboxylate; water soluble high molecular substances such as hyaluronic acid, collagen, mucosaccharides and chondroitin sulfate; and. . . molecular substances such as carboxyvinyl polymers and polyvinyl alcohol; and the like. Usable antioxydants include dibutylhydroxytoluene, butylhydroxyanisole, propyl gallate and **ascorbic acid**; chelating agents include disodium edetate, ethane hydroxy diphosphate, pyrophosphates, hexametaphosphates, citric acid, tartaric acid and gluconic acid; pH-adjusting agents include. . . vitamin B, vitamin C, vitamin D, vitamin E, vitamin F, vitamin K, vitamin P, vitamin U, carnitine, ferulic acid, .gamma.-oryzanol, **.alpha.-lipoic acid** and orotic acid and their derivatives; and **amino**

acids include glycine, **alanine**, **valine**, **leucine**, **isoleucine**, serine, threonine, **phenylalanine**, tyrosine, tryptophan, cystine, **cysteine**, methionine, proline, hydroxyproline, aspartic acid, glutamic acid, **arginine**, histidine and lysine and their derivatives. Optional components are not limited to those listed.

DETD . . . Magnolia bark, Cnidium, Bitter orange pool, Japanese angelica root, Ginger, Scutellaria, Gardenia, Artemisia vulgaris var. vulgatissima (and var. indica), Aloe, **Ginseng**, Cinnamon bark, Peony root, Japanese peppermint leaf, Scutellaria, Hoelen, Japanese iris, Schizandra nigra Maxim, Angelicae Dahuricae Radix (Pai-chi), Saffon, Cork. . .

DETD . . . water 3

Sodium ellagate	0.05%
Glycerin	3.0
Ethanol	6.0
Perfume	trace
Purified water	balance

Toilet water 4

A. Ethyl alcohol phase part

Ethyl alcohol	10.0%
POE(80) hardened castor oil	0.3
Tocopherol	0.1
Methylparaben	0.1
Perfume	appropriate amount

B. Water phase part

Potassium ellagate (treated pH 5)	0.05%
Glycerin	3.0
Purified water	balance

DETD

Emulsion 2

Ellagic acid	0.5%
Stearic acid	1.0
Cetanol	2.0
Vaseline	2.5
Squalane	4.0
L-**arginine**	1.0
Lipophilic type glycerin monostearate	1.0
Glycerin	2.0
Potassium hydroxide	0.1
Perfume	trace
Purified water	balance

Pack

A. Ethyl alcohol phase part

Ethyl alcohol	10.0%
Polyvinyl alcohol	15.0
Propylene glycol. . .	

CLM What is claimed is:

6. The method of claim 5, wherein the composition further contains a basic **amino acid** or monosaccharide in an amount of 0.001 to 30% by weight.

AN 91:102211 USPATFULL|

TI Agent containing an ellagic acid series compound for external application and use thereof|

IN Arima, Masatoshi, Odawara, Japan
Nishizawa, Hiroaki, Fujisawa, Japan
Takeuchi, Keiji, Tokyo, Japan
Deura, Hiroshi, Yotsukaidou, Japan
Ishida, Keiichiro, Tokyo, Japan

PA Lion Corporation, Tokyo, Japan (non-U.S. corporation)
PI US 5073545 19911217 <--
AI US 1988-202321 19880606 (7)
PRAI JP 1987-143507 19870609
JP 1988-70396 19880324
DT Utility|
EXNAM Primary Examiner: Brown, Johnnie R.; Assistant Examiner: Peselev, Elli|
LREP Burns, Doane, Swecker & Mathis|
CLMN Number of Claims: 10|
ECL Exemplary Claim: 1|
DRWN No Drawings
LN.CNT 620|
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 79 OF 82 USPATFULL
PI US 5032506 19910716 <--
DETD . . . dehydrogenase and showed that the enzyme reduces a wide variety of lipoic acid derivatives, for example: DL-lipoic acid, DL-lipoyl glycine, DL-lipoyl-beta-**alanine**, DL-lipoyl glycylglycine, DL-carboethoxy lipoanilide, DL-lipoanilide, and DL-lipoamide, in addition to potassium ferricyanide. The enzyme from an aerobic prokayrotes, for example. . .
DETD U.S. Pat. No. 4,247,633 describes the production of a dried, all-in-one reagent for the assay of **creatine** phosphokinase. This dried reagent contains: ADP, **creatine** phosphate, magnesium ions, glucose, hexokinase, NAD or NADP, INT, diaphorase, buffer, reduced glutathione, and AMP.
DETD . . . Pat. No. 4,215,197 describes the test means and method for creatinine determination. In this patent, creatinine is enzymatically hydrolyzed to **creatine**. The **creatine** is further enzymatically hydrolyzed to sarcosine and urea, and the sarcosine is enzymatically converted to formaldehyde and glycine with the. . .
DETD . . . discusses a reagent and method for the determination of lactate dehydrogenase. This invention uses pig heart diaphorase obtained from the **protein** fraction of a pig's heart insoluble in 1.6 to 2.8M ammonium sulfate by treating the insoluble **protein** fraction with 0.1-0.3% w/v polyethyleneimine, heating at 70 to 80 degrees C, absorption on a weakly acidic cation exchanger and. . .
DETD . . . (1969), which is incorporated herein by reference. Specifically included are those derivatives in which the lipoic acid is bonded to **amino acid** through an amide bond. The synthesis of many substituted lipoic acid analogs which are suitable for this invention, are disclosed. . .
DETD . . . for the dehydrogenase used in each specific example: glucose dehydrogenase, L-glutamic dehydrogenase, glyoxylate reductase, hydroxybutyrate dehydrogenase, polyol dehydrogenase, sorbital dehydrogenase, myo-**inositol** dehydrogenase, isocitrate dehydrogenase, 2-ketoglutarate dehydrogenase, **leucine** dehydrogenase, lipoamide dehydrogenase, malic dehydrogenase, malic enzyme, succinate semialdehyde oxldoreductase, 5-10-methylenetetrahydrofolate dehydrogenase, NADH peroxidase, cytochrome C reductase, octopine dehydrogenase, 3-phosphoglycerate dehydrogenase, dihydropteridine reductase, pyruvate dehydrogenase, sacharopine dehydrogenase, uridine-5'-diphos- phate dehydrogenase, xylulose reductase, 6-phosphogluconic dehydrogenase, **alanine** dehydrogenase, dihydrofolate reductase, glucose-6-phosphate dehydrogenase, hydroxyacyl CoA dehydrogenase, 1 acetate dehydrogenase, glycerophosphate dehydrogenase, glycerol dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, alcohol dehydrogenase, aldehyde. . .
DETD . . . in accordance with the invention can be measured are, but not limited to, various alcohols like methanol, ethanol, idiotol, sorbitol, **inositol**; organic acids, like malic acid; aldehydes like

formaldehyde, acetylaldehyde; sugars and **carbohydrates** like glucose, galactose and a variety of other organic compounds which are reactive to enzyme-catalyzed dehydrogenation or hydrogenation, like ketones, aminos (like **amino acids**), glycerol-3-phosphate, glycine, lactate, maleate and the like.

DETD . . . more commonly in a biological fluid sample. Of course, a very large number of such compounds are of interest including: **carbohydrates**--e.g. glucose, **amino acids**, **proteins**, alcohols, sugars, ketones. Illustrative are the following: biological fluids including serum, plasma, whole blood, urine, saliva, amniotic and cerebrospinal fluids,. . .

DETD The lipoic acid is replaced by 60 mM of DL-lipoamide beta-**alanine**. A like color intensity is obtained which can be read directly.

CLM What is claimed is:

20. The system of claim 19 wherein the lipoic acid compound is selected from the group consisting of DL-**alpha-lipoic acid**, DL-alpha-lipoamide, DL-lipoyl glycine, DL-dihydrolipoyl glycine, DL-lipoyl beta-**alanine**, DL-lipoyl glycylglycine, DL-carboethoxy lipoanilide, DL-lipoanilide and DL-dihydrolipoanalide.

28. The system of claim 22 wherein the organic analyte is selected from the group consisting of **carbohydrates**, polyalcohols and ketones.

48. The device of claim 47 wherein the organic analyte is selected from the group consisting of **carbohydrates**, polyalcohols and ketones and the biological medium is selected from the group consisting of blood, serum, saliva and urine.

AN 91:56845 USPATFULL|
TI Color control system|
IN Palmer, John L., Philadelphia, PA, United States
Timmerman, Marsha W., Allentown, PA, United States
PA Enzymatics, Inc., Horsham, PA, United States (U.S. corporation)
PI US 5032506 19910716 <--
AI US 1986-942414 19861216 (6)
DT Utility|
EXNAM Primary Examiner: Kepplinger, Esther L.; Assistant Examiner: Scheiner, Toni R.|
LREP Finnegan, Henderson, Farabow, Garrett & Dunner|
CLMN Number of Claims: 61|
ECL Exemplary Claim: 1|
DRWN 14 Drawing Figure(s); 13 Drawing Page(s)|
LN.CNT 1486|
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 80 OF 82 USPATFULL
PI US 4514387 19850430 <--
SUMM . . . action on blood plasma or blood cells. It is known that on the one hand, endotoxins may activate blood plasma **protein** systems, such as kinine and complement **protein** systems. On the other hand, they have a mitogenic effect on mononuclear leukocytes (B-cell mitogens); see Anderson et al. J.. . .

SUMM As a result of these findings, humoral serum **protein** preparations with chemotactic and/or chemokinetic activity on leukocytes have been prepared. However, these preparations have been neither molecularly homogeneous nor. . .

SUMM Thus, some of these **protein** preparations also induce a leukocytosis reaction in vivo; see B. Damerau et al., Naunyn-Schmiedberg's Arch. Pharmacol. 302 (1978), p. 45 to 50. Detailed investigations of the mechanisms of formation of humoral chemotaxins for leukocytes derived from serum-**proteins** have shown their relationship with anaphylatoxin activity which was detected by

Friedberger in 1910; see J. A. Jensen, in Ingram,. . .

SUMM More recently, using modern chromatographical preparation techniques, such biological active humoral trace **proteins** could be isolated and characterized in molcularly homogenous, crystalline and chemotactically acting form after about 5,000 to 20,000 fold purification; see J. H. Wissler, Eur. J. Immunol., vol. II, (1972), p. 73-96. These **protein** preparations have neither a leukokinetic activity nor can they mobilize and recruit leukocytes from the bone marrow into blood circulation.

SUMM It is these molecular-biological properties, i.e. the distinct cell and action specificity, in which the natural humoral leukotaxin **protein** preparations prepared and highly purified from contact-activated serum basically differ from less purified natural and,

especially, from synthetic low-molecular peptide. . .

SUMM . . . leukotaxin preparations. While the synthetic peptides indiscriminately activate cells to chemotaxis, chemokinesis, adhesion and aggregation, the specific natural humoral leukotaxin **protein** preparations only induce directional locomotion (chemotaxis) of leukocytes, without influencing their chemokinesis, adhesion, aggregations or phagocytosis responses.

SUMM All the mentioned and described preparations for influencing the chemokinesis and chemotaxis of leukocytes are humoral, serum **protein**-derived chemical substances. In addition, the existence of cellular (cell-secreted) chemotaxins has been shown. Furthermore, a migration-inhibiting activity of cellular origin. . .

SUMM The chemokinesins and chemotaxins of the invention have typical **protein proteins** and **protein** reactions (folin and biuret reactions). Their melting points is at approximately 200.degree. C. (decomposition in an air and oxygen-free atmosphere)..

.

SUMM . . . biologically specific activity. Where in the following text both groups of substances are meant, they are referred to as "mediator **proteins**" for the sake of brevity. It is their biological task to regulate the emigration of mature and juvenile blood leukocytes. The mediator **proteins** are not normal independent blood or serum components. Apart from many other hormones and mediators, they are formed in vitro. . . or in vivo upon accumulation of leukocytes at the site of inflammation. From this it is apparent that the mediator **proteins** of the invention differ in many of their biological and chemical properties from structural and functional properties of the bacterial. . .

SUMM The activity of the mediator **proteins** of the invention is measured in three different test systems. The first test is the direct microscopic observation of individual. . .

SUMM . . . proschemokinesins are involved. Where the activity is directed to a cell type in a selective or specific manner, the mediator **proteins** are named by definition, for instance "monapokinesin", "mono-proskinesin" or .-+.granulo-apokinesin" and "granulo-proskinesin".

This means that the corresponding chemokinesin reduces or. . .

SUMM . . . a chemokinesin which is produced by monocytes and specifically reduces the locomotion of granulocytes. Analogously, the monocyto-granuloproskinesin (MGPK) is a **protein** which is produced by monocytes and specifically increases the random locomotion of granulocytes. The lymphocyto-monoproskinesin (LMPK) is a chemokinesin which. . .

SUMM Apart from or in addition to the above-mentioned properties which the mediator **proteins** of the invention have in common, the LMAK has the following special properties:

SUMM molecular weight of the native **protein** (primary structure): approximately 14,000 dalton;

SUMM Apart from or in addition to the above-mentioned properties which the mediator **proteins** of the invention have in common, the MGK has

the following special properties:

SUMM molecular weight of the native **protein** (primary structure): approximately 9,000 dalton;

SUMM no **protein** quaternary structure in the form of physically bound peptide subunits: each of the native **proteins** consists of only one peptide unit;

SUMM Apart from or in addition to the above-mentioned properties which the mediator **proteins** of the invention have in common, the MGPK has the following special properties:

SUMM molecular weight of the native **protein** (primary structure): approximately 16,000 dalton;

SUMM Apart from or in addition to the above-mentioned properties which the mediator **proteins** of the invention have in common, the LMPK has the following special properties:

SUMM molecular weight of the native **protein** (primary structure): approximately 22,000 dalton;

SUMM Apart from or in addition to the above-mentioned properties which the mediator **proteins** of the invention have in common, MGT has the following special properties:

SUMM molecular weight of the native **protein** (primary structure): approximately 11,000 dalton;

SUMM Apart from or in addition to the above-mentioned properties which the mediator **proteins** of the invention have in common, GMT has the following special properties:

SUMM molecular weight of the native **protein** (primary structure): approximately 17,000 dalton;

SUMM Apart from and in addition to the above-mentioned properties which the mediator **proteins** of the invention have in common, MET has the following special properties:

SUMM molecular weight of the native **protein** (primary structure): approximately 5,000 dalton;

SUMM no **protein** quaternary structure in the form of physically bound peptide subunits: each of the native **proteins** consists of only one peptide unit;

SUMM Up to non-physiological concentrations of 10 .mu.mol/l, the mediator **proteins** of the invention have neither leucocytosis-inducing nor phagocytotic or mitosis-stimulating activities on neutrophil, eosinophil and mononuclear leukocytes of man, rabbit,. . .

SUMM FIGS. 1 to 7 show the UV absortion spectra of the highly purified mediator **proteins** LMAK, MGAK, MGPK, LMPK, MGT, GMT and MET in water at 20.degree. C. and extinction scale (0-100) E=0-2 at a. . .

SUMM . . . to these definitions, the LMAK-preparation is pyrogen-free and without febrile activity. This also applies to the other highly purified mediator **protein** preparations. This extremely sensitive criterion for contamination of **proteins** with bacterial endotoxins and other ubiquitous pyrogens demonstrates the great efficacy of the process of the purification of the cellular mediator **proteins** of the invention. It is an obvious parameter for the biological specificity of the mediator **proteins**.

SUMM The mediator **proteins** prepared and obtained according to the invention are valuable, endogeous substances. They can be used for specifically influencing the defence-state. . . and accumulation of leukocytes for inducing desired inflammatory reactions and controlling undesired ones, for instance in tumors. Moreover, the mediator **proteins** can be used for producing their antibodies which are also suitable to specifically influence leukocyte accumulation processes.

SUMM The mediator **proteins** of the invention are applied locally alone or as a mixture to mammalians, for instance man, in the form of. . .

SUMM Another subject matter of the invention is a process for the biotechnical preparation and isolation of mediator **proteins** from leukocytes and from inflamed tissue sites. It is characterized in

that either the leukocytes or the inflamed tissue are homogenized; or that leukocytes are cultured and the mediator **proteins** formed or liberated are isolated from the homogenates or from the supernatant culture solution.

SUMM . . . by intracellular structural constituents of leukocytes. Therefore, in the process of the invention, it is preferred to isolate the mediator **proteins** from the supernatant solution of the leukocyte culture. In principle, the leukocytes can be cultured in any leukocyte-compatible medium.

SUMM . . . normally are aqueous solutions which contain numerous different compounds. Main constituents of these culture media are salts, sugars and metabolites, **amino acids** and derivatives, nucleosides, vitamins, vitaminoids, coenzymes, steroids, antibiotics and other additives, such as tensides, heavy metal salts and indicator dyes.. . .

SUMM . . . for the maintenance of cellular functions. However, if the serum-containing culture solution is to be subjected to processes for isolating **proteins** (mediators) which are formed by culturing cells, the preparation of trace **protein** products is difficult for reasons of the multiplicity of compounds making up the complex mixture of serum added to the. . .

SUMM . . . the culture supernatant. The tensides, heavy metal salts and/or dyes contained therein may damage or irreversibly contaminate the trace mediator **proteins**.

SUMM . . . can be suitably used for the culture of leukocytes and the biotechnical preparation of cellular trace components, such as mediator **proteins**.

SUMM . . . is preferably used. It provides favourable conditions for cell culture and facilitates the preparation and isolation of the cellular mediator **proteins** from the culture supernatant.

SUMM . . . preferably used in this invention contains the normal groups of compounds, such as salts, sugars, polyols, uronic acids, and derivatives, **amino acids** and derivatives, nucleosides and nucleoside bases, vitamins, vitaminoids, phtyl derivatives, coenzymes and steroids in aqueous solution. It is characterized in. . .

SUMM . . . leukocyte culturing, the cell culture medium is preferably used without addition of serum. Instead, it contains at least one defined **protein**.

SUMM . . . invention, the synthetic, serum-free cell culture medium used in this invention may contain additional compounds, e.g. polyhydroxy compounds and sugars, **amino acids**, nucleosides, anionic compounds and/or vitamins which are not common in the known culture media. These compounds are useful in culturing. . .

SUMM . . . m

2	Potassium dihydrogenphosphate	0.2 m
3	Potassium chloride	5.0 m
4	Sodium chloride	120.0 m
5	Sodium sulfate	0.2 m
6	D-Glucose	5.0 m
7	L-**Ascorbic acid** (C)	0.2 m
8	Choline chloride	50.0.mu.
9	2-Deoxy-D-ribose	5.0.mu.
10	D-Galactose	0.5 m
11	D-Glucurono-.gamma.-lactone	0.1 m
12	Glycerol	50.0.mu.
13	Myo-**inositol**	0.5 m
14	Sodium acetate	0.2 m
15	Sodium citrate	50.0.mu.

16 Sodium pyruvate 0.1 m
17 D-Ribose 20.0.mu.
18 Succinic acid 0.1 m
19 Xylitol. . . 20.0.mu.
21 Calcium chloride 2.0 m
22 Magnesium chloride 1.0 m
23 Sodium hydrogencarbonate 10.0 m
24 Serum albumin (human) 7.7.mu.
25 L-Asparagine 0.1 m
26 L-**Glutamine** 1.0 m
27 Adenosine 50.0.mu.
28 4-Aminobenzoic acid 2.0.mu.
29 L-Aspartic acid 0.1 m
30 D-Biotine (Vitamin H) 1.0.mu.
31 Cytidine 50.0.mu.
32 L-Glutamic acid 0.1 m
33 L-**Isoleucine** 0.2 m
34 5-Methylcytosine 5.0.mu.
35 L-**Phenylalanine** 0.1 m
36 Riboflavine (B2) 1.0.mu.
37 Thymine (5-methyluracil) 5.0.mu.
38 L-Tryptophane 50.0.mu.
39 L-Tyrosine 0.1 m
40 Uracil 5.0.mu.
41 Uridine 20.0.mu.
42 L-**Leucine** 0.2 m
43 L-**Valine** 0.2 m
44 Thymidine 20.0.mu.
45 Water 55.4
46 Hydrogen ions (pH 7.1) 79.4 n
47 Oxygen (air saturation) 0.2 m
48 L-**Alanine** 0.2 m
49 L-**Arginine** 0.1 m
50 D,L-Carnithine chloride (BT) 50.0.mu.
51 L-Carnosine 5.0.mu.
52 L-**Cysteine** 0.2 m
53 L-Glutathione reduced 3.0.mu.
54 Glycine 0.2 m
55 L-Histidine 0.1 m
56 L-Hydroxyproline 10.0.mu.
57 L-Lysine-HCl 0.2 m
58 L-Methionine 0.1 m
59 D,L-Mevalolactone. . . Guanosine 20.0.mu.
77 Hypoxanthine 5.0.mu.
78 Rutin (Vitamin P) 5.0.mu.
79 Xanthine 5.0.mu.
80 Ethanol (60 .mu.l/l) 1.0 m
81 Cholesterol 1.0.mu.
82 Ergocalciferol (D2) 0.5.mu.
83 D,L-.**alpha**.-**Lipoic acid** 2.0.mu.
84 Menadione (K3) 0.2.mu.
85 D,L-.alpha.-**Tocopherol** acetate (E) 1.0.mu.
86 Coenzyme Q 10 ubiquinone 50 0.1.mu.
87 3-Phytylmenadione (K1) 0.2.mu.
88 Retinol acetate (A) 1.0.mu.
89 Linolenic acid (F) 5.0.mu.
90. . .

SUMM Certain types of the inventive mediator **proteins** are already obtained in satisfactory yields by normal culture of leukocytes or

certain leukocyte types. The GMT, for instance, is. . .

SUMM Other types of mediator **proteins** of the invention however, are are however only formed in small amounts by normal culture of leukocytes or certain leukocyte types. This applies for instance to the mediator **proteins** of mononuclear cells.

SUMM To terminate culturing, the leukocytes are centrifuged from the supernatant culture solution which is subsequently processed for the resulting mediator **proteins**. To avoid damaging the cells and thus contamination of the culture solution with cell particles, the culture is centrifuged at. . .

SUMM The supernatant culture solution freed from the cells contains the secretion products of the cultured leukocytes. These include the mediator **proteins** of the invention and a number of other **proteins** and other substances. Their concentration in the culture solution is approximately within the nanomolar range. Consequently, a yield of about. . . of the molecular efficiency of the cells, about 10.sup.14 leukocytes are necessary for obtaining a quantity of about 100 nmol **proteins**. This corresponds to about 1 mg of a mediator with the molecular weight of 10,000 dalton. This means that for. . .

SUMM Apart form leukocyte cultures, the mediator **proteins** of the invention can also be obtained from inflamed tissue sites. There, they are formed by the accumulation of leukocytes. . . tissue injuries. The inflamed tissue can be obtained in the usual manner and used for the preparation of the mediator **proteins**. Inflamed tissues are homogenized in buffer solution and soluble constituents or exudates are separated from insoluble structural components by means. . .

SUMM As shown above, the preparation and isolation of the mediator **proteins** of the invention requires the processing of a very large culture solution volume. Therefore, at the beginning of the purification. . . process effective reduction of the solution volume to be processed is necessary. In addition to the small amounts of the **proteins** produced, the culture solution contains the mixture of the components of the medium. Preferably in the first step of the purification process a separation of the formed **proteins** from the medium components with a concomitant reduction of the large volume of aqueous solution is achieved. This can be effected by selective salting-out precipitation of the **proteins** from the supernatant culture solution, for instance by adding a sulfate or a phosphate. In the following, the salting-out precipitation of **proteins** is exemplified by adding ammonium sulfate to the culture solution.

SUMM By saturation of the supernatant culture solution with ammonium sulfate, a major portion of the **proteins** formed is precipitated together with serum albumin present as medium component. The **proteins** precipitated are recovered e.g. by centrifugation. They are then separated into the individual components of the mixture as described below. Thereby, some mediator **proteins** are obtained. On the other hand, some other mediator **proteins** are salt-soluble and remain in the supernatant solution of the salting-out precipitation process. This supernatant also contains all soluble components of the medium. It is concentrated and the **proteins** obtained are processed in the manner described below.

SUMM If the **protein**-containing supernatant culture solution is saturated with ammonium sulfate, a major portion of **proteins** is precipitated. In this way, a **protein** mixture is obtained consisting of numerous different **proteins**. Their separation into the individual **protein** components is obviously laborious. Therefore, in a preferred embodiment of the inventive process the **protein** mixture of the supernatant culture solution is already separated into several fractions by the salting-out precipitation step. The separation into several crude **protein** fractions is possible, since groups of individual **proteins** precipitate at different ammonium sulfate concentrations. Preferably, in the process of

the invention, ammonium sulfate is therefore added stepwise to the culture solution up to a specific degree of saturation. Each fraction contains a group of **proteins**, the solubility product of which corresponds to the range of salt saturation. Hence, in the process according to the invention a crude separation into groups of **proteins** can be achieved in this first step by suitable choice of the saturation limits.

SUMM For instance, the supernatant culture solution is first brought to a 35%

saturation with ammonium sulfate. The **protein** precipitate obtained is separated off. The 35% saturation of the supernatant solution is then increased to 45% by further addition of ammonium sulfate. A **protein** precipitate is again formed which is separated off. Thereafter, the 45% salt-saturated supernatant solution is brought to a 90% ammonium sulfate saturation. The **protein** precipitate formed is again separated off. The supernatant solution of this precipitate is concentrated e.g. by dehydration dialysis or ultrafiltration.

SUMM The salting-out precipitation of **proteins** is preferably carried out at a temperature of about 0.degree. to 10.degree. C., especially of about 0.degree. to 4.degree. C.. . . 0.1 mol/l of phosphate buffer is preferably added prior to the salting-out precipitation. To maintain the redox potential of the **proteins** , **cysteine** is preferably added in an amount of 0.001 mol/l to all solutions throughout the process. The **protein** purification does not require sterile conditions.

SUMM After dissolution in a **protein**-compatible medium, the **proteins** obtained by salting-out precipitation can be directly subjected to purification and separation in the manner described below. The 90% salt-saturated. . .

SUMM The **protein** fractions obtained in the step described above contain the mediator **proteins** of the invention in admixture with numerous foreign **proteins**, e.g. other secreted **proteins**, in part serum albumins and in part CON. These foreign **proteins** form the major part of the constituents of this mixture. The mediator **proteins** must be further purified by a sequence of further purification steps. Foreign **proteins** must be removed to avoid interference with the molecular-biological specifity

of mediator **proteins**. In addition, mediator **proteins** themselves form a class of **protein** compounds which must be separated into individual, specifically acting structures.

SUMM In general, purification processes for **proteins** and other natural substances comprise sequences of combined separation techniques.

Subtle differences in molecular size, charge, form, structure stability and. . . Accordingly, a large number of combinations of various modifications of preparation techniques can be devised for the purification of a **protein**. The nature and the conditions of the preparation steps used, but also their sequential combination, are of paramount significance for. . .

SUMM For the purification of the individual **protein** fractions, a plurality of purification steps so far known in biochemistry can be used. Examples of such purification steps are:. . .

SUMM It is possible to remove a considerable amount of accompanying foreign **proteins** from mediator **proteins** by only one performance of these purification methods. However, **proteins** contained in the fractions tend to adhere together very strongly. Therefore, for example, in spite of different molecular weights of **proteins**, using molecular sieve filtration, no complete (ideal) separation of **protein** polyelectrolytes according to their exact molecular weight is obtained immediately. Hence it is necessary to

perform at least two of. . . process in accordance with the invention

uses three of the mentioned purification steps in sequence for the

purification of mediator **protein** activity from the **protein** fractions.

SUMM Molecular sieve filtration achieves separation of **proteins** according to their molecular weights. Since the bulk of the foreign **proteins** have molecular weights different from those of mediator **proteins** they can be separated off in this manner. A hydrophilic water-swelling molecular sieve as matrix is used for separation of the **proteins** by molecular weight. Examples of suitable molecular sieve matrices are dextrans cross-linked with epichlorohydrin (Sephadex), agaroses cross-linked with acrylamides (Ultrogels),. . .

SUMM . . . preparative molecular sieve chromatography, gel matrices with the largest particle size are used for maximum flow-through rates of mostly viscous **protein** solutions applied at reasonably low pressures. In analytical molecular sieve filtration the particle size ranges of the gel matrix are. . .

SUMM . . . the gel used must in all cases be higher than about 10,000 daltons to allow a volume distribution of mediator **proteins** between the stationary gel matrix phase and the mobile aqueous buffer phase.

SUMM For molecular sieve filtration, the **proteins** are applied to the molecular sieve after dissolution in a **protein**-compatible liquid. A special example of a suitable solvent is 0.003 mol/l sodium-potassium phosphate solution containing 0.3 mol/l NaCl and 0.001 mol/l **cysteine** and having a pH of 7.4. After filtration, the mediator **protein**-containing fractions are concentrated in the manner described below and optionally subjected to a further purification step.

SUMM . . . 10. A special example of such a buffer solution is 0.01 mol/l tris-HCl containing 0.04 mol/l NaCl and 0.001 mol/l **cysteine** and having a pH value of 8.0.

SUMM The anion exchanger is added to the **protein** fraction in an amount sufficient for complete adsorption of the mediator **proteins** and of the other positively adsorbing accompanying **proteins**. Two volume parts of swollen anion exchanger per volume of concentrated **protein** solution are normally sufficient. The reaction can be carried out either as chromatographic process or as an easy and fast batch adsorption technique. In the latter case, the supernatant liquid containing negatively adsorbed **proteins** is separated from the anion exchanger which is charged with the positively adsorbed mediator **proteins** or other **proteins**, e.g. by filtration in a chromatographic column, by decantation or centrifugation. The charged anion exchanger is freed from adhering negatively. . .

SUMM The anion exchanger on which the mediator **proteins** and other **proteins** are adsorbed and which is freed from the negatively adsorbed compounds is eluted with a **protein**-compatible aqueous salt solution having an ionic strength higher than 0.04 mol/l NaCl and a pH of between 4.0 and 10.0.. . . is a 2.0 mol/l NaCl solution buffered to a pH of 6.5 with 0.01 mol/l piperazine-HCl and containing 0.001 mol/l **cysteine**.

SUMM If the anion exchange reaction is carried out as a chromatographic process, elution of the mediator **proteins** and other positively adsorbed **proteins** can also be done by a linear NaCl concentration gradient.

SUMM Examples of cation exchange matrices suitable for the purification of the **protein** fraction are dextrans crosslinked with epichlorohydrin (Sephadex) or cellulose matrices carrying functional groups with cation exchange capacity. These can be. . . To facilitate the charge process and to approach more ideal equilibria conditions prior to treatment with the cation exchanger the **protein** fractions should be diluted with a **protein**-compatible salt solution having a maximum ionic strength equivalent to 0.04 mol/l NaCl. This salt solution can be used at the. . . a salt solution for this

purpose is a 0.001 mol/l potassium phosphate-acetate buffer containing 0.04 mol/l NaCl and 0.001 mol/l **cysteine** and having a pH of 4 to 6. This cation-exchange reaction may be performed as a chromatographic process, or technically. . .

SUMM The swollen cation exchanger is added to the **protein** fraction in a quantity sufficient to adsorb it. As a rule, about 2 volume parts of swollen ion exchanger per volume part of **protein** solution is sufficient for this purpose. The supernatant is then separated from the cation exchanger charged with **proteins**, for example by decantation or centrifugation. The charged cation exchanger is freed from adhering, negatively adsorbed compounds by washing with. . .

SUMM The washed **protein**-charged cation exchanger is now eluted with a **protein**-compatible aqueous salt solution. A salt solution of high ionic strength with a pH of about 4 to 10 is preferably. . .

SUMM For chromatography on hydroxyapatite, salts, e.g. ammonium sulfate and especially phosphates, possibly present from preceding steps are removed from the **protein** solution, preferably by dialysis or ultrafiltration at membranes with an exclusion limit of 500 daltons prior to the application of the **proteins** to hydroxyapatite. Apart fom viscosity increase by accompanying salts, however, only the phosphate concentration of the **protein** solution is critical for the chromatography on hydroxyapatite. The mediator **proteins** are eluted by a potassium phosphate concentration gradient which is preferably linear. The mediator **protein** containing fractions are collected and then concentrated in the manner described below.

SUMM The use of hydroxyapatite is of essential significance for the structure-conserving isolation of pure mediator **proteins**. However, in general, for technical and economic reasons, considerable difficulties arise from chromatography of larger volumes of **protein** solutions on hydroxyapatite columns. On the one hand, larger **protein** amounts contribute to the strong tendency of hydroxyapatite to clog, thus becoming unusable as stationary matrix in chromatography. On the. . . economical. For these reasons, in the process of the invention, the separation of a large part of the accompanying foreign **proteins** by appropriate biotechnical purification steps from the mediator **protein**-containing **protein** fractions is preferred for considerably reducing the volume of the **protein** solution prior to its chromatography on hydroxyapatite.

SUMM In the zone precipitation chromatography (cf. J. Porath, Nature, vol. 196 (1962); p. 47-48), residual **protein** contaminations in the mediator **proteins** are separated by salting-out fractionation of the **proteins** by means and along a salt concentration gradient. The basic principle of separation of **proteins** in zone precipitation chromatography are different, structure-related, reversible solubility characteristics of **proteins**. They belong to the most sensitive molecular separation criteria and are often used for demonstration of molecular homogeneity of a **protein**. Two variants of this technique for development of the chromatogram are known: Fractional precipitation zone chromatography and fractional elution zone. . . types of techniques may have selective advantages in specific cases as described for fractional precipitation and fractional elution methods in **protein** separation. Temperature and pH, column characteristics can all be varied within relatively wide limits.

SUMM . . . should be greater than about 10:1. A ratio of 30 to 100:1 and especially of about 50:1 is preferred. All **protein**-compatible salts having salting-out properties for **proteins** are suitable. Examples of such salts are sodium-potassium phosphate, ammonium sulfate, and sodium sulfate. Ammonium sulfate is preferred.

SUMM The salt concentration gradient can have any desired shape provided that salting-out criteria of **proteins** achieve **protein** separation. Linear concentration gradients are preferred, especially as

ascendent linear concentration gradient from 25 to 100% ammonium sulfate
saturation. The. . .
SUMM . . . eluate is recycled onto the same column with fixed separation limits. In this way, the separation length of the migrating **protein** distribution bands are differentially extended.
Alternatively, in cascade molecular sieve filtration, distribution equilibria are disturbed by continuous transfer of the. . .
SUMM Between the above-described purification steps, and if necessary at any stage for special purposes, **protein** solutions can be separated and freed from unwanted salts and water as well as concomitantly concentrated. The concentration (separation of a major portion of aqueous salt solution of the **protein**) can be achieved in different ways. Dehydration dialysis or ultrafiltration against **protein**-compatible liquid, preferably a sodium potassium phosphate buffer, are such methods. Dehydration dialysis is carried out preferably against polyethylene glycol (molecular. . . preferably 500
daltons. Ultrafiltration is preferably achieved at membranes with an exclusion limit of about 500 daltons. Small amounts of **protein** precipitates formed are removed by intermediary centrifugation to result
in a clear **protein** solution. A desalting molecular sieve filtration on matrices with appropriate separation and exclusion limits can as well be used for. . .
SUMM To prevent sulfhydryl group oxidation, about 0.001 mol/l of **cysteine** is preferably added to **protein** solutions throughout.
SUMM In the molecular sieve filtration purification steps about 0.4 mol/l ammonium sulfate is preferably added to the **protein** solution. In contrast to higher concentrations of this salt, at this concentration
ammonium sulfate exerts a strong salting-in effect on **proteins**. Thus, **proteins** are better kept in solution during the molecular sieve filtration. Moreover, ammonium sulfate prevents growth of microorganisms and inhibits certain enzymes. Hence, it contributes to
stabilization of the mediator **protein** structure which is important when chromatography is performed at higher temperature (above about 20.degree. C.) and under nonsterile conditions.
SUMM Mediator **proteins** which can be salted out are preferably completely precipitated alone or together with accompanying **proteins** by adding ammonium sulfate up to a concentration of about 3.25 to 3.7 mol/l (80 to 90% saturation). For this. . . kept between 4 and 9 and the temperature up to 40.degree. C., preferably between 0.degree. and 8.degree. C. The mediator **protein**-containing **protein** precipitate is separated from the **protein**-free supernatant solution by filtration, decantation or centrifugation. Unless otherwise stated, centrifugation is preferably carried out at least at 10,000.times.g for. . . be carried out in two
stages, at lower forces in the first stage for removal of the bulk of precipitated **proteins**; and then, for the supernatant of the first stage containing residual fine **protein** particles at higher forces, e.g. 20,000 to 50,000.times.g, by flow-through centrifugation.
SUMM . . . temperature and pH conditions during performance of the purification steps are not particularly critical. If the native conformation of the **protein** is to be preserved, an optimum temperature range is about 0.degree. to 8.degree. C., and preferably about 0.degree. to 4.degree.. . .
SUMM The mediator **proteins** obtained can be stored in a buffered physiological saline, e.g. in 0.0015 mol/l sodium-potassium phosphate solution containing 0.15 mol/l (0.9 w/v%) NaCl, 0.001 mol/l **cysteine** and having a pH of 7.4. After usual sterilization by filtration (pore diameter 0.2 .mu.m), the **protein** preparation

remains native and biologically active at room temperature for at least 200 h or frozen at -25.degree. C. for at least 5 years. This stability of the **protein** can be considered, among others, to be one of the criteria of molecular homogeneity. Mediator **protein** solutions are safely stored at temperatures of between -20.degree. and +50.degree. C. in the presence of 2.0 to 3.6 mol/l ammonium sulfate (50 to 90% saturation). At this high osmotic pressure mediator **protein** solutions are protected against infection and degradation by microorganisms and bacterial growth. For their physiological, therapeutical and any other use, the mediator **proteins** are again freed from salts by dialysis or ultrafiltration against an appropriate saline as described above.

SUMM The invention will now be given in detail by examples describing the isolation of the mediator **protein** preparation starting from leukocytes of porcine blood. However, the invention is not restricted to this embodiment. Leukocytes and inflamed tissues. . .

DETD . . . supernatant are described. All process steps are carried out at 0.degree. to 8.degree. C. in the presence of 0.001 mol/l **cysteine**, unless otherwise specified. The centrifugation is carried out in the manner described, either as a one or two step procedure. . .

DETD . . . techniques. The functional viability of cells is measured by their motility and their ability to respond to chemokinetic and chemotactic **proteins**. Mitoses are determined by chromosome count. The morphological viability of the cells after their biotechnical culturing is 95%. The entire. . .

DETD . . . particles. The resultant clear supernatant culture solution which has a total volume of 1000 liters and contains about 1,400 g **protein** as well as other macromolecules and salts is directly subjected to salting-out fractionation with ammonium sulfate (A2). Unless otherwise stated,. . .

DETD A2. First purification step (salting-out fractionation): Preparation of crude **protein** concentrate fractions

DETD . . . 6.7 is added to the supernatant culture solution (A 1) up to a final concentration of 0.1 mol/l. Furthermore, solid L-**cysteine** is added up to a concentration of 0.001 mol/l.

DETD . . . saturation of ammonium sulfate by addition of 199 g of ammonium sulfate/l solution. During the addition, the pH-value of the **protein** solution is continuously controlled and maintained at 6.7 by the addition of 2 n ammonia. Part of the **proteins** is precipitated from the solution. The **protein** precipitate formed is separated from the supernatant containing salt-soluble **proteins** by centrifugation for 1 hour at 10,000.times.g. The precipitated crude **protein** fraction I is obtained as ammonium sulfate-containing **protein** sludge which contains about 100 g **protein**. This crude **protein** concentrate fraction I may separately be processed for its constituents according to the procedure described below for the crude **protein** concentrate fraction III.

DETD . . . adjusted to 45% saturation of ammonium sulfate by adding 60 g of ammonium sulfate/l solution. The pH value of the **protein** solution is continuously controlled and maintained constant at 6.7 by 2 n ammonia. Another protion of **proteins** is pecipitated from the solution. The **protein** precipitate is separated from the supernatant containing salt-soluble **proteins** by centrifugation for 1 hour at 10,000.times.g. The precipitated crude **protein** concentrate fraction II is obtained as ammonium sulfate-containing **protein** sludge, the **protein** content of which is about 60 g. This crude **protein** concentrate fraction II may be processed separately for its constituents, according to the procedure described below for the crude **protein** concentrate fraction III.

DETD . . . adjusted to 90% saturation of ammonium sulfate by adding 323 g of ammonium sulfate/l of solution. The pH-value of the **protein** solution is again continuously controlled and maintained constant at 6.7

by 2 n ammonia. Another portion of the **proteins** is precipitated from the solution. The **protein** precipitate is separated from the supernatant containing salt-soluble **proteins** by centrifugation for 1 hour at 10,000.times.g. The precipitated crude **protein** concentrate fraction III is obtained as ammonium sulfate-containing **protein** sludge the **protein** content of which is approximately 1,080 g. This fraction also contains the bulk of the serum albumin as component of the culture medium. This crude **protein** concentrate fraction III contains the mediator **proteins** of the invention and is processed according to the procedure described below. The 90% salt saturated supernatant fraction IV of the crude fraction III contains 160 g of salt-soluble **proteins** and other macro molecules (>500 daltons). It may also be processed for its constituents.

DETD A.3. Fine purification of mediator **proteins** in the crude **protein** concentrate fraction III

DETD The crude **protein** concentrate fraction III obtained above (A 2) is dissolved in a minimum volume of buffer solution B (0.01 mol/l of tris-HCl solution containing 0.04 mol/l NaCl and 0.001 mol/l **cysteine** and having a pH value of 8.0). The resultant slightly turbid solution (20 l) is clarified by centrifugation and then. . .

DETD The column has four times the volume of the **protein** solution and a length-to-diameter ratio of 10:1. The gel column is then washed with the above-mentioned adsorption buffer solution B. . .

DETD For elution of the chemokinesins and the adsorbed **proteins**, the charged ion exchanger gel is eluted with a NaCl-concentration gradient during 2 days. The gradient is linearly ascending from 0.04 to 2.0 mol/l NaCl, whereas the pH value, the tris/HCl and the **cysteine** concentrations are maintained constant. The same shape of gradient is then used for lowering the pH from 8 to 6.5. . . further elution of the compounds. It is made up by 0.01 mol/l piperacine-HCl-buffer containing 2.0 mol/l NaCl and 0.001 mol/l **cysteine** and having the pH 6.5.

DETD After concentration of the **proteins** in the fractions (A.3.1) by salting-out precipitation with ammonium sulfate, the **protein** precipitate containing either chemokinesins or chemotaxins is dissolved in a minimum volume of buffer solution C (0.003 mol/l sodium-potassium phosphate containing 0.3 mol/l NaCl and 0.001 mol/l **cysteine** and having a pH value of 7.4). After removal of a small amount of insoluble compounds by centrifugation, the solution. . . particle size 60 to 160 .mu.m) for preparative molecular sieve filtration. The column has 10 times the volume of the **protein** solution and a length-to-diameter ratio of 20:1. The column is then eluted with an upward flow (3 cm/h) of the. . . for chemotaxins, the fraction with the separation limits of 20,000 and 3,000 dalton are collected. For the concentration of the **proteins**, the fractions are lyophilized and ultrafiltrated at a membrane with the exclusion limit of 500 dalton or are adjusted to an ammonium sulfate concentration of 3.7 mol/l. In this case, the **protein** precipitates are separated from the supernatant by centrifugation and further processed as described below (A.3.3)

DETD The resultant chemokinesins or chemotaxins-containing **protein** precipitates (A 3.2) are dissolved in 1.5 volume parts of buffer solution D (0.01 mol/l sodium-potassium phosphate, 0.04 mol/l NaCl, 0.001 mol/l **cysteine**, pH 6.0). The solutions are centrifuged at 10,000.times.g for 1 hour for removal of a small amount of insoluble material.

DETD The column has four times the volume of the **protein** solution and a length-to-diameter ratio of 10:1. The gel column is then washed with the above-mentioned adsorption buffer solution D,. . .

DETD For elution of the mediator **proteins** and the adsorbed **proteins**, the charged ion exchange gel is eluted with an

NaCl-concentration gradient during 2 days. The gradient is linearly ascending from 0.04 to 2.0 mol/l NaCl whereas the pH-value and the phosphate and **cysteine** concentrations are maintained constant. For further elution, the same shape of gradient is then used for increasing the phosphate concentration from 0.01 to 0.5 mol/l at a pH of

8.0, whereas the NaCl (2 mol/l) and **cysteine** concentrations are kept constant.

DETD The chemokinesins or chemotaxins-containing **protein** precipitates (A.3.3) are dissolved in a minimum volume of 0.0001 mol/l sodium-potassium phosphate buffer solution E containing 0.001 mol/l **cysteine** and having a pH of 7.20. The solutions are then desalted with this buffer by molecular sieve filtration, ultrafiltration or. . .

DETD The clear chemokinesins or chemotaxins-containing **protein** solutions obtained are separately applied to a column of hydroxyapatite. The length-to-diameter ratio of the column is 10:1 and it has four times the volume of the **protein** volume to be applied. The column has been equilibrated with the mentioned buffer E used in an amount five times. . .

DETD The negatively adsorbed **proteins** are washed out with the buffer solution E used for equilibrating the column. The elution of the mediator **protein**-containing fractions is carried out with a phosphate concentration gradient for 4 days. The gradient is linearly ascending from 0.0001 mol/l to 0.5 mol/l sodium-potassium phosphate having a constant pH value of 7.4 and constant **cysteine** concentration.

DETD The different mediator **proteins** are separated in this step. LMAK is eluted at an average phosphate concentration of about 0.04 mol/l, MGAK at about. . . mol/l and GMT at about 0.2 mol/l. The elution gradient is measured and controlled by means of conductivity. The mediator **protein**-containing fractions are concentrated in the usual manner and further processed as described below (A.3.5).

DETD The mediator **protein**-containing fractions (A.3.4) are dissolved in 0.1 mol/l sodium-potassium phosphate solution F containing 0.1 mol/l NaCl, 0.001 mol/l **cysteine** and 1 mol/l ammonium sulfate and having a pH value of 7.4. The resultant solution is applied at a temperature. . . The length-to-diameter ratio of the column is 50:1, the column volume is 100 times higher than the **protein** solution volume to be applied. The flow rate is 2 cm/h.

DETD The elution is carried out with the above-mentioned sodium-potassium phosphate solution F containing 1 mol/l of ammonium sulfate. The mediator **protein**-containing fractions which are eluted at 65% (LMAK), 77% (MGAK), 72% (MGPK), 62% (LMPK), 70% (MGT), 57% (GMT) and 80% (MET) ammonium sulfate saturation, respectively, are collected. The **proteins** are concentrated in the usual manner and further processed as described below (A.3.6).

DETD The mediator **protein**-containing fractions (A.3.5) are dissolved in buffer C (0.003 mol/l sodium-potassium phosphate containing 0.3 mol/l NaCl and 0.001 mol/l casteine and. . .

DETD . . . AcA 44 having a particle size of 60 to 140 .mu.m. The column has 50 times the volume of the **protein** solution and a length-to-diameter ratio of 50:1. The elution is carried out with the mentioned buffer C. The eluates are. . . dalton (MGAK), 19,000 dalton (MGPK), 25,000 dalton (LMPK), 13,000 dalton (MGT), 20,000 dalton (GMT), or 8,000 dalton (MET). After usual **protein** concentration, approximately 3 mg of LMAK, 5 mg of MGAK, 5 mg of MGPK, 4 mg of LMPK, 6 mg. . .

DETD Preparation of Mediator **Proteins** from Supernatants of Cultures of Viable Lymphocytes

DETD Preparation of Mediator **Proteins** from Supernatants of Cultures of Viable Monocytes

DETD Preparation of Mediator **Proteins** from Inflamed Tissue Sites

DETD . . . used. The heart muscle tissue is ground at 0.degree.-4.degree. C. 0.05 mol/l sodium potassium phosphate buffer solution containing 0.001 mol/l **cysteine** and having a pH of 6.8 is added in a quantity three times the amount of the tissue. The resultant. . . .

DETD The mediator **protein**-containing clear supernatant **protein** solution is then subjected to fractional salting-out precipitation with ammonium sulfate according to example A. The resultant **protein** fraction III is then processed as described in example A. The yields, as compared to example A, are about 50% with the **proteins** from monocytes and granulocytes and only about 10% with the **proteins** from lymphocytes.

DETD Preparation of Mediator **Proteins** from Leukocyte Homogenates

DETD . . . homogenate of 500 g of leukocytes is prepared as shown in example D for muscle tissue. The isolation of the **protein** mediators contained in the leukocytes is performed according to example A. The leukocytes cultured without stimulation contain only relatively small. . . .

CLM What is claimed is:

. . . of macrophages (monocytes) in vitro; effective threshold dose in vitro: -2 nmol/l; (b) physico-chemical properties: molecular weight of the native **protein** (primary structure): approximately 14,000 dalton; absorption spectrum (UV, visible and near IR-range) as given in FIG. 1; extinction coefficients according. . . .

. . . motility of granulocytes in vitro; effective threshold dose in vitro; <1 nmol/l; (b) physico-chemical properties: molecular weight of the native **protein** (primary structure): approximately 9,000 dalton; no **protein** quaternary structure in the form of physically bound peptide subunits: each of the native **proteins** consists of only one peptide unit; constant temperature coefficient of solubility in ammonium sulfate solutions between -10.degree. C. and +50.degree.. . . .

. . . motility of granulocytes in vitro; effective threshold dose in vitro: <2 nmol/l; (b) physico-chemical properties: molecular weight of the native **protein** (primary structure): approximately 16,000 dalton; absorption spectrum (UV, visible and near IR-range) as given in FIG. 3; extinction coefficient according. . . .

. . . of macrophages (monocytes) in vitro; effective threshold dose in vitro: <10 nmol/l; (b) physico-chemical properties: molecular weight of the native **protein** (primary structure): approximately 22,000 dalton; adsorption spectrum (UV, visible and near IR-range) as given in FIG. 4; extinction coefficient according. . . .

. . . cell-induced angiogenesis and inflammation reaction; effective threshold dose in vitro: <0.5 nmol/l; (b) physico-chemical properties: molecular weight of the native **protein** (primary structure): approximately 11,000 dalton; absorption spectrum (UV visible and near IR-range) as given in FIG. 5; extinction coefficient according. . . .

. . . cell-induced angiogenesis and inflammation reaction; effective threshold dose in vitro: <10 nmol/l; (b) physico-chemical properties: molecular weight of the native **protein** (primary structure): approximately 17,000 dalton; absorption spectrum (UV, visible and near IR-range) as given in FIG. 6; extinction coefficient according. . . .

. . . of eosinophilic leukocytes in situ; effective threshold dose in vitro; <5 nmol/l; (b) physico-chemical properties: molecular weight of the native **protein** (primary structure): approximately 5,000 dalton; no **protein** quaternary structure in the form of physically bound peptide subunits: each of the native **proteins** consists of only one peptide unit; absorption spectrum (UV, visible and near IR-range) as given in FIG. 7; extinction coefficient. . . .

. . . said medium to yield a culture solution; (c) adding a sufficient

amount of a suitable salt to precipitate a first **protein** fraction from said culture solution; (d) separating said first **protein** portion from said solution; (e) concentrating said solution to obtain a second **protein** fraction therefrom; (f) separately purifying said first and second **protein** fractions by molecular sieve filtration, anion and cation exchange chromatography,
chromatography on hydroxypapatite, zone precipitation chromatography, or
recycling molecular sieve. . .

. . . claim 15, wherein the leukocytes are cultured in a fully synthetic cell culture medium containing serum albumin as the only **protein** .

26. The process according to claim 25, wherein ammonium sulfate is used for precipitating the **proteins**.

27. The process according to claim 26, wherein the ammonium sulfate concentration of the culture solution is stepwise increased and the **proteins** precipitated after each ammonium sulfate addition are separated, thereby yielding several crude **protein** fractions having differing solubilities at different ammonium sulfate concentration.

29. The process according to claim 25, wherein the supernatant after separation of the **protein** precipitate is concentrated by ultrafiltration or dialysis.

. . . yield a culture solution; (d) adding ammonium sulfate to the culture solution up to 90% saturation in order to precipitate **proteins** contained therein; (e) separating the precipitated **proteins** from the ammonium sulfate containing supernatant; (f) redissolving the precipitated **proteins**; (g) purifying said **proteins** by anion exchange chromatography, preparative molecular sieve filtration, cation exchange chromatography, chromatography on hydroxyapatite, zone precipitation chromatography and recycling molecular. . .

. . . sufficient ammonium sulfate to the culture solution to provide an ammonium sulfate concentration of up to 90% saturation to precipitate **proteins** contained therein; (f) separating the precipitated **proteins** from the ammonium sulfate containing supernatant; (g) redissolving the precipitated **proteins**; (h) purifying said **proteins** by anion exchange chromatography, preparative molecular sieve filtration, cation exchange chromatography, chromatography on hydroxyapatite, zone precipitation chromatography, and recycling molecular. . .

. . . form a culture solution; (e) adding ammonium sulfate to the culture solution up to 90% saturation in order to precipitate **proteins** contained therein; (f) separating the precipitated **proteins** from the ammonium sulfate containing supernatant; (g) redissolving the precipitated **proteins**; (h) purifying said **proteins** by anion exchange chromatography, preparative molecular sieve filtration, cation exchange chromatography, chromatography on hyroxyapatite, zone precipitstion chromatography and recycling molecular. . .

. . . culturing to yield a culture solution; (e) adding ammonium sulfate to
the culture solution up to 90% saturation to precipitate **proteins** contained therein; (f) separating the precipitated **proteins** from the ammonium sulfate containing supernatant; (g) redissolving said precipitated **proteins**; (h) purifying said **proteins** by anion exchange chromatography, preparative molecular sieve filtration, cation exchange chromatography, chromatography on hydroxyapatite, zone precipitation chromatography and recycling molecular. . .

. . . culturing to yield a culture solution; (e) adding ammonium sulfate to

the culture solution up to 90% saturation to precipitate **proteins** concentrated therein; (f) separating the precipitated **proteins** from the ammonium sulfate containing supernatant; (g) redissolving said precipitated **proteins**; (h) purifying said **proteins** by anion exchange chromatography, preparative molecular sieve filtration, cation exchange chromatography, chromatography on hydroxyapatite, zone prcipitation chromatography and recycling molecular. . .

. . . culture solution; (e) adding ammonium sulfate to the culture solution to achieve up to 90% saturation in order to precipitate **proteins** contained therein; (f) separating the precipitated **proteins** from the ammonium sulfate containing supernatant; (g) redissolving said **proteins**; (h) purifying said **proteins** by anion exchange chromatography, preparative molecular sieve filtration, cation exchange chromatography, chromatography on hydroxyapatite, zone precipitation chromatography and recycling molecular. . .

. . . solution; (e) adding ammonium sulfate to the culture solution to achieve up to a 90% saturation in order to precipitate **proteins** contained therein; (f) separating the precipitated **proteins** from the ammonium sulfate containing supernatant; (g) redissolving the precipitated **proteins**; (h) purifying said **proteins** by anion exchange chromatography, preparative molecular sieve filtration, cation exchange chromatography, chromatography on hydroxyapatite, zone precipitation chromatography and recycling molecular. . .

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TI Chemokinesins and chemotaxins of leukocytes and inflamed tissues|
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EXNAM Primary Examiner: Hazel, Blondel|
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CAS INDEXING IS AVAILABLE FOR THIS PATENT.

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PI US 4512971 19850423 <--
SUMM . . . the inflammation and tissue regeneration processes. The mediators are formed either by limited and regulated proteolysis of plasma and serum **protein** factors as humoral mediators; or they are liberated by active secretion and/or cell lysis from cells and tissues as cellular. . . blood and are present in very minute concentrations only. Experimental evidence shows that only up to 5,000 of such mediator **protein** molecules can be maintained in a steady state equilibrium by a cell in the mitotic cycle in its surrounding medium.
SUMM The mitogens of the invention have typical **protein** properties and **protein** reactions (folin and biuret reactions). Their melting point is approximately 200.degree. C. (decomposition in an air and oxygen-free atmosphere).
SUMM The mitogens of the invention are cellular inflammatory **protein** mediators with topochemically and biologically specific activity. It is their biological task to stimulate the division and differentiation of some. . .
SUMM molecular weight of the native **protein** (primary structure); approximately 25,000 dalton;
SUMM molecular weight of the native **protein** (primary structure): approximately 85,000 daltons;

SUMM molecular weight of the native **protein** (primary structure): approximately 13,000 daltons;

SUMM molecular weight of the native **protein** (primary structure): approximately 17,000 dalton;

SUMM . . . without febrile activity. This also applies to the other highly purified mitogen preparations. This extremely sensitive criterion for contamination of **proteins** with bacterial endotoxins and other ubiquitous pyrogens demonstrates the great efficacy of the process of the purification of the cellular. . .

SUMM . . . normally are aqueous solutions which contain numerous different compounds. Main constituents of these culture media are salts, sugars and metabolites, **amino acids** and derivatives, nucleosides, vitamins, vitaminoids, coenzymes, steroids and other additives, such as tensides, heavy metal salts and indicator dyes. Special. . . for the maintenance of cellular functions. However, if the serum-containing culture solution is to be subjected to processes for isolating **proteins** (mediators) which are formed by culturing cells, the preparation of trace **protein** products is difficult for reasons of the multipilicity of compounds making up the complex mixture of serum added to the. . .

SUMM . . . the culture supernatant. The tensides, heavy metal salts and/or dyes contained therein may damage or irreversibly contaminate the trace mediator **proteins**.

SUMM . . . is preferably used. It provides favourable conditions for cell culture and facilitates the preparation and isolation of the cellular mitogen **proteins** from the culture supernatant.

SUMM . . . preferably used in this invention contains the normal groups of compounds, such as salts, sugars, polyols, uronic acids, and derivatives, **amino acids** and derivatives, nucleosides and nucleoside bases, vitamins, vitaminoids, phytyl derivatives, coenzymes and steroids in aqueous solution. It is characterized in. . .

SUMM . . . leukocyte culturing, the cell culture medium is preferably used without addition of serum. Instead, it contains at least one defined **protein**.

SUMM . . . invention, the synthetic, serum-free cell culture medium used in this invention may contain additional compounds, e.g. polyhydroxy compounds and sugars, **amino acids**, nucleosides, anionic compounds and/or vitamins which are not common in the known culture media. These compounds are useful in culturing. . .

SUMM . . . 3 Potassium chloride 5.0 m

4 Sodium chloride 120.0 m

5 Sodium sulfate 0.2 m

6 D-Glucose 5.0 m

7 L-**Ascorbic acid** (C) 0.2 m

8 Choline chloride 50.0.mu.

9 2-Deoxy-D-ribose 5.0.mu.

10 D-Galactose 0.5 m

11 D-Glucurono-.gamma.-lactone 0.1 m

12 Glycerol 50.0.mu.

13 Myo-**inositol** 0.5 m

14 Sodium acetate 0.2 m

15 Sodium citrate 50.0.mu.

16 Sodium pyruvate 0.1 m

17 D-Ribose 20.0.mu.

18 Succinic acid 0.1 m

19 Xylitol. . . 20.0.mu.

21 Calcium chloride 2.0 m

22	Magnesium chloride	1.0 m
23	Sodium hydrogencarbonate	10.0 m
24	Serum albumin (human)	7.7.mu.
25	L-Asparagine	0.1 m
26	L-**Glutamine**	1.0 m
27	Adenosine	50.0.mu.
28	4-Aminobenzoic acid	2.0.mu.
29	L-Aspartic acid	0.1 m
30	D-Biotine (Vitamin H)	1.0.mu.
31	Cytidine	50.0.mu.
32	L-Glutamic acid	0.1 m
33	L-**Isoleucine**	0.2 m
34	5-Methylcytosine	5.0.mu.
35	L-**Phenylalanine**	0.1 m
36	Riboflavine (B2)	1.0.mu.
37	Thymine (5-methyluracil)	5.0.mu.
38	L-Tryptophane	50.0.mu.
39	L-Tyrosine	0.1 m
40	Uracil	5.0.mu.
41	Uridine	20.0.mu.
42	L-**Leucine**	0.2 m
43	L-**Valine**	0.2 m
44	Thymidine	20.0.mu.
45	Water	55.4
46	Hydrogen ions (pH 7.1)	79.4 n
47	Oxygen (air saturation)	0.2 m
48	L-**Alanine**	0.2 m
49	L-**Arginine**	0.1 m
50	D,L-Carnithine chloride (BT)	50.0.mu.
51	L-Carnosine	5.0.mu.
52	L-**Cysteine**	0.2 m
53	L-Glutathione reduced	3.0.mu.
54	Glycine	0.2 m
55	L-Histidine	0.1 m
56	L-Hydroxyproline	10.0.mu.
57	L-Lysine-HCl	0.2 m
58	L-Methionine	0.1 m
59	D,L-Mevalolactone	
. . .	Guanosine	20.0.mu.
77	Hypoxanthine	5.0.mu.
78	Rutin (Vitamin P)	5.0.mu.
79	Xanthine	5.0.mu.
80	Ethanol (60 .mu.l/l)	1.0 m
81	Cholesterol	1.0.mu.
82	Ergocalciferol (D2)	0.5.mu.
83	D,L-.**alpha.-Lipoic acid**	2.0.mu.
84	Menadione (K3)	0.2.mu.
85	D,L-.alpha.-**Tocopherol** acetate (E)	1.0.mu.
86	Coenzyme Q 10 ubiquinone 50	0.1.mu.

87 3-Phytylmenadione (K1)
0.2.mu.
88 Retinol acetate (A)
1.0.mu.
89 Linolenic acid (F)
5.0.mu.
90. . .
SUMM . . . contains the secretion products of the cultured leukocytes. These include the mitogens of the invention and a number of other **proteins** and other substances. Their concentration in the culture solution is approximately within the nanomolar range. Consequently, a yield of about. . . of the molecular efficiency of the cells, about 10.sup.14 leukocytes are necessary for obtaining a quantity of about 100 nmol **proteins**. This corresponds to about 1 mg of a mediator with the molecular weight of 10,000 dalton. This means that for. . .
SUMM . . . process effective reduction of the solution volume to be processed is necessary. In addition to the small amounts of the **proteins** produced, the culture solution contains the mixture of the components of the medium. Preferably, in the first step of the purification process a separation of the formed **proteins** from the medium components with a concomitant reduction of the large volume of aqueous solution is achieved. This can be effected by selective salting-out precipitation of the **proteins** from the supernatant culture solution, for instance by adding a sulfate or a phosphate. In the following, the salting-out precipitation of **proteins** is exemplified by adding ammonium sulfate to the culture solution.
SUMM By saturation of the supernatant culture solution with ammonium sulfate,
a major portion of the **proteins** formed is precipitated together with serum albumin present as medium component. The **proteins** precipitated are recovered e.g. by centrifugation. They are then separated into the individual components of the mixture as described below.. . . of the salting-out precipitation process. This supernatant also contains all soluble components of the medium. It is concentrated and the **proteins** obtained are processed in the manner described below.
SUMM If the **protein**-containing supernatant culture solution is saturated with ammonium sulfate, a major portion of **proteins** is precipitated. In this way, a **protein** mixture is obtained consisting of numerous different **proteins**. Their separation into the individual **protein** components is obviously laborious. Therefore, in a preferred embodiment of the inventive process the **protein** mixture of the supernatant culture solution is already separated into several fractions by the salting-out precipitation step. The separation into several crude **protein** fractions is possible, since groups of individual **proteins** precipitate at different ammonium sulfate concentrations. Preferably, in the process of
the invention, ammonium sulfate is therefore added stepwise to the culture solution up to a specific degree of saturation. Each fraction contains a group of **proteins**, the solubility product of which corresponds to the range of salt saturation. Hence, in the process according to the invention a crude separation into groups of **proteins** can be achieved in this first step by suitable choice of the saturation limits.
SUMM For instance, the supernatant culture solution is first brought to a 35%
saturation with ammonium sulfate. The **protein** precipitate obtained is separated off. The 35% saturation of the supernatant solution is then increased to 45% by further addition of ammonium sulfate. A **protein** precipitate is again formed which is separated off. Thereafter, the 45% salt-saturated supernatant solution is brought to a 90% ammonium sulfate saturation. The **protein** precipitate formed is again separated off. The supernatant solution of this precipitate is concentrated e.g. by dehydration dialysis or

ultrafiltration.

SUMM The salting-out precipitation of **proteins** is preferably carried out at a temperature of about 0.degree. to 10.degree. C., especially of about 0.degree. to 4.degree. C.. . . . 0.1 mol/l of phosphate buffer is preferably added prior to the salting-out precipitation. To maintain the redox potential of the **proteins** , **cysteine** is preferably added in an amount of 0.001 mol/l to all solutions throughout the process. The **protein** purification does not require sterile conditions.

SUMM After dissolution in a **protein**-compatible medium, the **proteins** obtained by salting-out precipitation can be directly subjected to purification and separation in the manner described below. The 90% salt-saturated. . .

SUMM The **protein** fractions obtained in the step described above contain the mitogens of the invention in admixture with numerous foreign **proteins**, e.g. other secreted **proteins**, in part serum albumins and in part CON. These foreign **proteins** form the major part of the constituents of this mixture. The mitogens must be further purified by a sequence of further purification steps. Foreign **proteins** must be removed to avoid interference with the molecular-biological specifity of mitogens. In addition, mitogens themselves form a class of **protein** compounds which must be separated into individual, specifically acting structures.

SUMM In general, purification processes for **proteins** and other natural substances comprise sequences of combined separation techniques.

Subtle differences in molecular size, charge, form, structure stability and. . . Accordingly, a large number of combinations of various modifications of preparation techniques can be devised for the purification of a **protein**. The nature and the conditions of the preparation steps used, but also their sequential combination, are of paramount significance for. . .

SUMM For the purification of the individual **protein** fractions, a plurality of purification steps so far known in biochemistry can be used. Examples of such purification steps are:. . .

SUMM It is possible to remove a considerable amount of accompanying foreign **proteins** from mitogens by only one performance of these purification methods. However, **proteins** contained in the fractions tend to adhere together very strongly. Therefore, for example, in spite of different molecular weights of **proteins**, using molecular sieve filtration, no complete (ideal) separation of **protein** polyelectrolytes according to their exact molecular weight is obtained immediately. Hence it is necessary to perform at least two of. . . with the invention uses three of the mentioned purification steps in sequence for the purification of mitogen activity from the **protein** fractions.

SUMM Molecular sieve filtration achieves separation of **proteins** according to their molecular weights. Since the bulk of the foreign **proteins** have molecular weights different from those of mitogens they can be separated off in this manner. A hydrophilic water-swelling molecular sieve as matrix is used for separation of the **proteins** by molecular weight. Examples of suitable molecular sieve matrices are dextrans cross-linked with epichlorohydrin (Sephadex), agaroses cross-linked with acrylamides (Ultrogels),. . .

SUMM . . . molecular sieve chromatography, gel matrices with the largest possible particle size are used for maximum flow-through rates of mostly viscous **protein** solutions applied at reasonably low pressures. In analytical molecular sieve filtration the particle size ranges of the gel matrix are. . .

SUMM For molecular sieve filtration, the **proteins** are applied to the molecular sieve after dissolution in a **protein**-compatible liquid. A special example of a suitable solvent is 0.003 mol/l

sodium-potassium phosphate solution containing 0.3 mol/l NaCl and 0.001 mol/l **cysteine** and having a pH of 7.4. After filtration, the mitogen-containing fractions are concentrated in the manner described below and optionally. . .

SUMM . . . 10. A special example of such a buffer solution is 0.01 mol/l tris-HCl containing 0.04 mol/l NaCl and 0.001 mol/l **cysteine** and having a pH value of 8.0.

SUMM The anion exchanger is added to the **protein** fraction in an amount sufficient for complete adsorption of the mitogens and of the other positively adsorbing accompanying **proteins**. Two volume parts of swollen anion exchanger per volume of concentrated **protein** solution are normally sufficient. The reaction can be carried out either as chromatographic process or as an easy and fast batch adsorption technique. In the latter case, the supernatant liquid containing negatively adsorbed **proteins** is separated from the anion exchanger which is charged with the positively adsorbed mitogens or other **proteins**, e.g. by filtration in a chromatographic column, by decantation or centrifugation. The charged anion exchanger is freed from adhering negatively. . .

SUMM The anion exchanger on which mitogens and other **proteins** are adsorbed and which is freed from the negatively adsorbed compounds is eluted with a **protein**-compatible aqueous salt solution having an ionic strength higher than 0.04 mol/l NaCl and a pH of between 4.0 and 10.0.. . . is a 2.0 mol/l NaCl solution buffered to a pH of 6.5 with 0.01 mol/l piperazine-HCl and containing 0.001 mol/l **cysteine**.

SUMM If the anion exchange reaction is carried out as a chromatographic process, elution of the mitogens and other positively adsorbed **proteins** can also be done by a linear NaCl concentration gradient.

SUMM Examples of cation exchange matrices suitable for the purification of the **protein** fraction are dextrans crosslinked with epichlorohydrin (Sephadex) or cellulose matrices carrying functional groups with cation exchange capacity. These can be. . . To facilitate the charge process and to approach more ideal equilibria conditions prior to treatment with the cation exchanger the **protein** fractions should be diluted with a **protein**-compatible salt solution having a maximum ionic strength equivalent to 0.04 mol/l NaCl. This salt solution can be used at the. . . a salt solution for this purpose is a 0.001 mol/l potassium phosphate-acetate buffer containing 0.04 mol/l NaCl and 0.001 mol/l **cysteine** and having a pH of 4 to 6. This cation-exchange reaction may be performed as a chromatographic process, or technically. . .

SUMM The swollen cation exchanger is added to the **protein** fraction in a quantity sufficient to adsorb it. As a rule, about 2 volume parts of swollen ion exchanger per volume part of **protein** solution is sufficient for this purpose. The supernatant is then separated from the cation exchanger charged with **proteins**, for example by decantation or centrifugation. The charged cation exchanger is free from adhereing, negative adsorbed compounds by washing with. . .

SUMM The washed **protein**-charged cation exchanger is now eluted with a **protein**-compatible aqueous salt solution. A salt solution of high ionic strength with a pH of about 4 to 10 is preferably. . .

SUMM For chromatography on hydroxyapatite, salts, e.g. ammonium sulfate and especially phosphates, possibly present from preceding steps are removed from the **protein** solution, preferably by dialysis or ultrafiltration at membranes with an exclusion limit of 500 daltons prior to the application of the **proteins** to hydroxyapatite. Apart from viscosity increase by accompanying salts, however, only the phosphate concentration of the **protein** solution is critical for the chromatography on hydroxyapatite. The mitogens are eluted by a potassium phosphate concentration gradient which is. . .

SUMM . . . of pure mitogens. However, in general, for technical and economic reasons, considerable difficulties arise from chromatography of larger volumes of **protein** solutions on hydroxyapatite columns. On the one hand, larger **protein** amounts contribute to the strong tendency of hydroxyapatite to clog, thus becoming unusable as stationary matrix in chromatography. On the. . . economical. For these reasons, in the process of the invention, the separation of a large part of the accompanying foreign **proteins** by appropriate biotechnical purification steps from the mitogen-containing **protein** fractions is preferred for considerably reducing the volume of the **protein** solution prior to its chromatography on hydroxyapatite.

SUMM In the zone precipitation chromatography (cf. J. Porath, Nature, vol. 196 (1962); p. 47-48), residual **protein** contaminations in the mitogens are separated by salting-out fractionation of the **proteins** by means and along a salt concentration gradient. The basic principle of separation of **proteins** in zone precipitation chromatography are different, structure-related, reversible solubility characteristics of **proteins**. They belong to the most sensitve molecular separation criteria and are often used for demonstration of molecular homogeneity of a **protein**. Two variants of this technique for development of the chromatogram are known: Fractional precipitation zone chromatography and fractional elution zone. . . types of techniques may have selective advantages in specific cases as described for fractional precipitation and fractional elution methods in **protein** separation. Temperature and pH, column characteristics can all be varied within relatively wide limits.

SUMM . . . should be greater than about 10:1. A ratio of 30 to 100:1 and especially of about 50:1 is preferred. All **protein**-compatible salts having salting-out properties for **proteins** are suitable. Examples of such salts are sodium-potassium phosphate, ammonium sulfate, and sodium sulfate. Ammonium sulfate is preferred.

SUMM The salt concentration gradient can have any desired shape provided that salting-out criteria of **proteins** achieve **protein** separation. Linear concentration gradients are preferred, especailly an ascendent linear concentration gradient from 25 to 100% ammonium sulfate saturation. The. . .

SUMM . . . eluate is recycled onto the same column with fixed separation limits. In this way, the separation length of the migrating **protein** distribution bands are differentially extended. Alternatively, in cascade molecular sieve filtration, distribution equilibria are disturbed by continuous transfer of the. . .

SUMM Between the above-described purification steps, and if necessary at any stage for special purposes, **protein** solutions can be separated and freed from unwanted salts and water as well as concomitantly concentrated. The concentration (separation of a major portion of aqueous salt solution of the **protein**) can be achieved in different ways. Dehydration dialysis or ultrafiltration against **protein**-compatible liquid, preferably a sodium potassium phosphate buffer, are such methods. Dehydration dialysis is carried out preferably against polyethylene glycol (molecular. . . preferably 500 daltons. Ultrafiltration is preferably achieved at membranes with an exclusion limit of about 500 daltons. Small amounts of **protein** precipitates formed are removed by intermediary centrifugation to result in a clear **protein** solution. A desalting molecular sieve filtration on matrices with appropriate separation and exclusion limits can as well be used for. . .

SUMM To prevent sulfhydryl group oxidation, about 0.001 mol/l of **cysteine** is preferably added to **protein** solutions

throughout.

SUMM In the molecular sieve filtration purification steps about 0.4 mol/l ammonium sulfate is preferably added to the **protein** solution. In contrast to higher concentrations of this salt, at this concentration ammonium sulfate exerts a strong salting-in effect on **proteins**. Thus, **proteins** are better kept in solution during the molecular sieve filtration. Moreover, ammonium sulfate prevents growth of microorganisms and inhibits certain. . .

SUMM Mitogens which can be salted out are preferably completely precipitated alone or together with accompanying **proteins** by adding ammonium sulfate up to a concentration of about 3.25 to 3.7 mol/l (80 to 90% saturation). For this. . . kept between 4 and 9 and the temperature up to 40.degree. C., preferably between 0.degree. and 8.degree. C. The mitogen-containing **protein** precipitate is separated from the **protein**-free supernatant solution by filtration, decantation or centrifugation. Unless otherwise staed, centrifugation is preferably carried out at least at 10,000.times.g for. . . be carried out in two stages, at lower forces in the first stage for removal of the bulk of precipitated **proteins**; and then, for the supernatant of the first stage containing residual fine **protein** particles at higher forces, e.g. 20,000 to 50,000.times.g, by flow-through centrifugation.

SUMM . . . temperature and pH conditions during performance of the purification steps are not particularly critical. If the native conformation of the **protein** is to be preserved, an optimum temperature range is about 0.degree. to 8.degree. C., and preferably about 0.degree. to 4.degree.. . .

SUMM . . . in a buffered physiological saline, e.g. in 0.0015 mol/l sodium-potassium phosphate solution containing 0.15 mol/l (0.9 w/v%) NaCl, 0.001 mol/l **cysteine** and having a pH of 7.4. After usual sterilization by filtration (pore diameter 0.2 .mu.m), the **protein** preparation remains native and biologically active at room temperature for at least 200 h or frozen at -25.degree. C. for at least 5 years. This stability of the **protein** can be considered, among others, to be one of the criteria of molecular homogeneity. Mitogen solutions are safely stored at. . .

SUMM The invention will now be given in detail by examples describing the isolation of the mitogen **protein** preparation starting from leukocytes of porcine blood. However, the invention is not restricted to this embodiment. Leukocytes and inflamed tissues. . .

DETD . . . supernatant are described. All process steps are carried out at 0.degree. to 8.degree. C. in the presence of 0.001 mol/l **cysteine**, unless otherwise specified. The centrifugation is carried out in the manner described, either as a one or two step procedure. . .

DETD . . . techniques. The functional viability of cells is measured by their motility and their ability to respond to chemokinetic and chemotactic **proteins**. Mitoses are determined by chromosome count. The morphological viability of the cells after their biotechnical culturing is 95%. The entire. . .

DETD . . . particles. The resultant clear supernatant culture solution which has a total volume of 1000 liters and contains about 1,400 g **protein** as well as other macromolecules and salts is directly subjected to salting-out fractionation with ammonium sulfate (A2). Unless otherwise stated,. . .

DETD A.2. First purification step (salting-out fractionation): Preparation of crude **protein** concentrate fractions.

DETD . . . of 6.7 is added to the supernatant culture solution (A1) up to a final concentration of 0.1 mol/l. Furthermore, solid L-

cysteine is added up to a concentration of 0.001 mol/l.

DETD . . . saturation of ammonium sulfate by addition of 199 g of ammonium sulfate/l solution. During the addition, the pH-value of the **protein** solution is continuously controlled and maintained at 6.7 by the addition of 2 n ammonia. Part of the **proteins** is precipitated from the solution. The **protein** precipitate formed is separated from the supernatant containing salt-soluble **proteins** by centrifugation for 1 hour at 10,000.times.g. The precipitated crude **protein** fraction I is obtained as ammonium sulfate-containing **protein** sludge which contains about 100 g **protein**. This crude **protein** concentrate fraction I may separately be processed for its constituents according to the procedure described below for the crude **protein** concentrate fraction III.

DETD . . . adjusted to 45% saturation of ammonium sulfate by adding 60 g of ammonium sulfate/l solution. The pH value of the **protein** solution is continuously controlled and maintained constant at 6.7 by 2 n ammonia. Another portion of **proteins** is precipitated from the solution. The **protein** precipitate is separated from the supernatant containing salt-soluble **proteins** by centrifugation for 1 hours at 10,000.times.g. The precipitated crude **protein** concentrate fraction II is obtained as ammonium sulfate-containing **protein** sludge, the **protein** content of which is about 60 g. This crude **protein** concentrate fraction II may be processed separately for its constituents, according to the procedure described below for the crude **protein** concentrate fraction III.

DETD . . . adjusted to 90% saturation of ammonium sulfate by adding 323 g of ammonium sulfate/l of solution. The pH-value of the **protein** solution is again continuously controlled and maintained constant at 6.7 by 2 n ammonia. Another portion of the **proteins** is precipitated from the solution. The **protein** precipitate is separated from the supernatant containing salt-soluble **proteins** by centrifugation for 1 hour at 10,000.times.g. The precipitated crude **protein** concentrate fraction III is obtained as ammonium sulfate-containing **protein** sludge the **protein** content of which is approximately 1,080 g. This fraction also contains the bulk of the serum albumin as component of the culture medium. This crude **protein** concentrate fraction III is processed for the contained mitogens MBG, GBG, MHM and LLM according to the procedure described below. The 90% salt saturated supernatant fraction IV of the crude fraction III contains 160 g of salt-solution **proteins** and other macro-molecules (>500 daltons). It may also be processed for its constituents.

DETD A.3. Fine purification of mitogens in the crude **protein** concentrate fraction III

DETD The crude **protein** concentrate fraction III obtained above (A2) is dissolved in a minimum volume of buffer solution B (0.01 mol/l of tris-HCl solution containing 0.04 mol/l NaCl and 0.001 mol/l **cysteine** and having a pH value of 8.0). The resultant slightly turbid solution (20 l) is clarified by centrifugation and then. . .

DETD The column has four times the volume of the **protein** solution and a length-to-diameter ratio of 10:1. The gel column is then washed with the above-mentioned adsorption buffer solution B. . .

DETD For elution of the mitogens and the adsorbed **proteins**, the charged ion exchanger gel is eluted with a NaCl-concentration gradient during 2 days. The gradient is linearly ascending from 0.04 to 2.0 mol/l NaCl, whereas the pH value, the tris/HCl and the **cysteine** concentrations are maintained constant. The same shape of gradient is then used for lowering the pH from 8 to 6.5. . . further elution of the compounds. It is made up by 0.01 mol/l piperacine-HCl-buffer containing 2.0 mol/l NaCl and 0.001 mol/l **cysteine** and having the pH 6.5.

DETD After concentration of the **proteins** in the fractions (A.3.1) by salting-out precipitation with ammonium sulfate, the **protein** precipitate containing either MBG,GBG,MHM or LLM is dissolved in a minimum volume of buffer solution C (0.003 mol/l sodium-potassium phosphate containing 0.3 mol/l NaCl and 0.001 mol/l **cysteine** and having a pH value of 7.4). After removal of a small amount of insoluble compounds by centrifugation, the solution. . . particle size 60 to 160 .mu.m) for preparative molecular sieve filtration. The column has 10 times the volume of the **protein** solution and a length-to-diameter ratio of 20:1. The column is then eluted with an upward flow (3 cm/h) of the. . . for LLM, the fraction with the separation limits of 14,000 and 20,000 dalton are collected. For the concentration of the **proteins**, the fractions are lyophilized, and ultrafiltered at a membrane with the exclusion limit of 500 daltons or are adjusted to an ammonium sulfate concentration of 3.7 mol/l. In this case, the **protein** precipitates are separated from the supernatant by centrifugation and further processed as described below (A.3.3).

DETD The resultant MBG, GBG, MHM or LLM-containing **protein** precipitates (A 3.2) are dissolved in 1.5 volume parts of buffer solution D (0.01 mol/l sodium-potassium phosphate, 0.04 mol/l NaCl, 0.001 mol/l **cysteine**, pH 6.0). The solutions are centrifuged at 10,000.times.g for 1 hour for removal of a small amount of insoluble material.

DETD The column has four times the volume of the **protein** solution and a length-to-diameter ratio of 10:1. The gel column is then washed with the above-mentioned adsorption buffer solution D,. . .

DETD For elution of GBG and the adsorbed **proteins**, the charged ion exchange gel is eluted with an NaCl-concentration gradient during 2 days. The gradient is linearly ascending from 0.04 to 2.0 mol/l NaCl whereas the pH-value and the phosphate and **cysteine** concentrations are maintained constant. For further elution, the same shape of gradient is then used for increasing the phosphate concentration from 0.01 to 0.5 mol/l at a pH of 8.0, whereas the NaCl (2 mol/l) and **cysteine** concentrations are kept constant.

DETD The mitogen-containing **protein** precipitates (A.3.3) are dissolved in a minimum volume of 0.0001 mol/l sodium-potassium phosphate buffer solution E containing 0.001 mol/l **cysteine** and having a pH of 7.20. The solutions are then desalted with this buffer by molecular sieve filtration, ultrafiltration or. . .

DETD The clear MBG, GBG, MHM or LLM-containing **protein** solutions obtained are separately applied to a column of hydroxyapatite. The length-to-diameter ratio of the column is 10:1 and it has four times the volume of the **protein** volume to be applied. The column has been equilibrated with the mentioned buffer E used in an amount five times. . .

DETD The negatively adsorbed **proteins** are washed out with the buffer solution E used for equilibrating the column. The elution of the MBG, GBG, MHM. . . is linearly ascending from 0.0001 mol/l to 0.5 mol/l sodium-potassium phosphate having a constant pH value of 7.4 and constant **cysteine** concentration. MBG is eluted at an average phosphate concentration of about 0.003 mol/l, GBG at about 0.1 mol/l, MHM at. . .

DETD The mitogen-containing fractions (A.3.4.) are dissolved in 0.1 mol/l sodium-potassium phosphate solution F containing 0.1 mol/l NaCl, 0.001 mol/l **cysteine** and 1 mol/l ammonium sulfate and having a pH value of 7.4. The resultant solution is applied at a temperature. . .

DETD The length-to-diameter ratio of the column is 50:1, the column volume is 100 times higher than the **protein** solution volume to be applied. The flow rate is 2 cm/h.

DETD . . . which are eluted at 72% (MBG), 52% (GBG), 65% (MHM) and 61%

(LLM) ammonium sulfate saturation, respectively, are collected. The **proteins** are concentrated in the usual manner and further processed as described below (A.3.6.).

DETD . . . AcA 44 having a particle size of 60 to 140 .mu.m. The column has 50 times the volume of the **protein** solution and a length-to-diameter ratio of 50:1. The elution is carried out with the mentioned buffer C. The eluates are. . . at separation limits of either 30,000 dalton (MBG), 100,000 dalton (GBG), 20,000 dalton (MHM) or 24,000 dalton (LLM). After usual **protein** concentration, approximately 6 mg of MBG, 8 mg of GBG, 6 mg of MHM and 5 mg of LLM are.

. .

DETD The mitogen-containing clear supernatant **protein** solution is then subjected to fractional salting-out precipitation with ammonium sulfate according to example A. The resultant **protein** fraction III is processed as described in example A. From the 500 g of tissue, mitogens are obtained in a. . .

CLM What is claimed is:

. . . differentiation of bone marrow leukocytes; effective threshold dose in vitro <50 pmol/l (b) physico-chemical properties: molecular weight of the native **protein** (primary structure); approximately 25,000 dalton; insoluble in an ammonium sulfate solution at 90% saturation (3.6 mol/l); absorption spectrum (UV, visible. . .

. . . differentiation of bone marrow leucocytes; effective threshold dose in vitro: <5 nmol/l (b) physico-chemical properties: molecular weight of the native **protein** (primary structure): approximately 85,000 dalton; insoluble in an ammonium sulfate solution at 90% saturation (3.6 mol/l); absorption spectrum (UV, visible. . .

. . . of mitosis of peritoneal macrophages; effective threshold dose in vitro <1 nmol/l (b) physico-chemical properties: molecular weight of the native **protein** (primary structure): approximately 13,000 dalton; insoluble in a 90% saturated ammonium sulfate solution (3.6 mol/l); absorption spectrum (UV, visible and. . .

. . . of mitosis of peripheral lymphocytes; effective threshold dose in vitro: <0,5 nmol/l (b) physico-chemical properties: molecular weight of the native **protein** (primary structure): approximately 17,000 dalton; insoluble in a 90% saturated ammonium sulfate solution (3.6 mol/l); absorption spectrum (UV, visible and. . .

. . . said medium to yield a culture solution; (d) adding a sufficient amount of a suitable salt to precipitate a first **protein** fraction from solution; (e) separating said first **protein** portion from solution; (f) adding a further amount of the salt to the solution to precipitate a second **protein** fraction therefrom; (g) separately purifying said first and second **protein** fractions by molecular sieve filtration, anion and cation exchange chromatography, chromatography on hydroxyapatite, zone precipitation chromatography, and recycling molecular sieve. . .

. . . claim 10, wherein the leukocytes are cultured in a fully synthetic cell culture medium containing serum albumin as the only **protein** .

20. The process according to claim 19, wherein ammonium sulfate is used for precipitating the **proteins.**

21. The process according to claim 20, wherein the ammonium sulfate concentration of the culture solution is stepwise increased and the **proteins** precipitated after each ammonium sulfate addition are separated, thereby yielding several crude **protein** fractions having differing solubilities at different ammonium sulfate concentrations.

23. The process according to claim 19, wherein the supernatant liquid after separation of the **protein** precipitate is concentrated by ultrafiltration or dialysis.

. . . solution; (d) adding ammonium sulfate to the culture solution to achieve up to a 90% saturation in order to precipitate **proteins** contained therein; (d) separating the precipitated **proteins** from the ammonium sulfate-containing supernatant; (f) redissolving said precipitated **proteins**; (g) purifying said **proteins** by anion exchange chromatography, preparative molecular seive filtration, cation exchange chromatography, chromatography on hydroxyapatite, zone precipitation chromatography and recycling molecular sieve filtration for removing the accompanying foreign **proteins**; and (h) isolating the substantially pure monocytoblastogen from the eluate of the recycling molecular sieve filtration by adding ammonium sulfate. . .

. . . to the culture solution to provide an ammonium sulfate concentration of up to a 90% saturation in order to precipitate **proteins** contained therein; (f) separating the precipitated **proteins** from the ammonium sulfate-containing supernatant; (g) redissolving said precipitated **proteins**; (h) purifying said **proteins** by anion exchange chromatography preparative molecular sieve filtration, cation exchange chromatography, chromatography on hydroxyapatite, zone precipitation chromatography, and recycling molecular. . .

. . . solution; (e) adding ammonium sulfate to the culture solution to achieve up to a 90% saturation in order to precipitate **proteins** contained therein; (f) separating the precipitated **proteins** from the ammonium sulfate containing supernatant; (g) redissolving said precipitated **proteins**; (h) purifying said **proteins** by anion exchange chromatography, preparative molecular sieve filtration, cation exchange chromatography, chromatography on hydroxyapatite, zone precipitation chromatography and recycling molecular sieve filtration for removing accompanying foreign **proteins**; and (i) isolating the highly purified monocytohistiomitogen from the eluate of the recycling molecular sieve filtration by adding up to. . .

. . . a culture solution; (e) adding ammonium sulfate to the culture solution up to a 90% saturation in order to precipitate **proteins** concentrated therein; (f) separating the precipitated **proteins** from the ammonium sulfate-containing supernatant; (g) redissolving said **proteins**; (h) purifying said **proteins** by anion exchange chromatography, preparative molecular sieve filtration, cation exchange chromatography, chromatography on hydroxyapatite, zone precipitation chromatography and recycling molecular sieve filtration for removing accompanying foreign **proteins**; and (i) isolating highly purified lymphocytolymphomitogen from the eluate of the recycling molecular sieve filtration by adding ammonium sulfate up. . .

AN 85:23877 USPATFULL|
TI Mitogens of leukocytes and inflamed tissues|
IN Wissler, Josef H., Bad Nauheim, Germany, Federal Republic of
PA Max Planck Gesellschaft Zur Forderung der Wissenschaften, Gottingen, Germany, Federal Republic of (non-U.S. corporation)
PI US 4512971 19850423 <--
AI US 1982-358098 19820315 (6)
PRAI DE 1981-3110611 19810318
DT Utility|
EXNAM Primary Examiner: Hazel, Blondel|
LREP Cooper, Dunham, Clark, Griffin & Moran|
CLMN Number of Claims: 37|
ECL Exemplary Claim: 1|
DRWN 7 Drawing Figure(s); 7 Drawing Page(s)|
LN.CNT 1583|
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 82 OF 82 USPATFULL
PI US 3697647 19721010 <--

DETD Erythritol	.+-.	Melibiose	++
Adonitol	.+-.	Maltose	+++
D-Sorbitol	+	Sucrose	+
L-**Inositol**	+++	Lactose	+++
D-Mannitol	+++ +	Raffinose	+
Dulcitol	+	Trehalose	+ + +
D-Xylose	+ + +	Salicin	+. . .
DETD Erythritol	.+-.	D-Maltose	+++
Adonitol	.+-.	Sucrose	.+-.
D-Sorbitol	.+-.	Lactose	+ + +
i-**Inositol**	+++	Raffinose	.+-.
D-Mannitol	+ + +	Salicin	+ + +
Dulcitol	.+-.	Aesculin	+
D-Xylose	+ + +	Inulin. . .	

DETD . . . addition, the drinking water or feedstuff for the livestock can
also contain antibiotics such as aureomycin, Mikamycin, Oleandomycin, Penicillin, Tetracyclin, **amino acids** such as glutamic acid, aspartic acid, **leucine**, lysine, tryptophane, **valine**, serine, proline, glucine, **alanine**, **isoleucine**, **phenylalanine**, argine, methionine, threonine, or their salts, vitamins such as Vitamin B.sub.1, Vitamin B.sub.2, Vitamin B.sub.6, Vitamin B.sub.12,, Vitamin C, Vitamin D, biotin, folic acid, Vitamin K, Vitamin E, Vitamin P, **inositol**, orotic acid, .**alpha**.-**lipoic acid**, etc.

AN 72:51391 USPATFULL
TI FEED CONTAINING ENDURACIDIN
IN Matsuoka, Toshiro, Suita, Japan
Takeda, Keinosuke, Kyoto, Japan
Goto, Minoru, Kyoto, Japan
Miayake, Akira, Nishinomiya, Japan
PA Takeda Chemical Industries, Ltd., Higashi-ku, Osaka, Japan (non-U.S. corporation)
PI US 3697647 19721010 <--
AI US 1968-700385 19680125 (4)
PRAI JP 1967-4408 19670125
DT Utility
EXNAM Primary Examiner: Goldberg, Jerome D.
LREP Wenderoth, Lind & Ponack
CLMN Number of Claims: 9
DRWN No Drawings
LN.CNT 909
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

has been on the market for at least three yrs.

Longevity Clinic of La Jolla

Growing Older without Getting Old.

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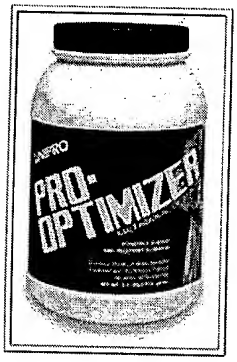

PRO ... OPTIMIZER™
Powerful Anabolic Post-Workout Recovery Drink
24 Grams of Whey Protein Hydrolysate Per Serving
High in Branched Chain Amino Acids

Pro ... Optimizer contains high biological value amino acids from whey protein hydrolysate (enzymatically predigested lactalbumin), with ratio-balanced carbohydrates and a comprehensive micronutrient formula, providing building blocks and energy sources helpful for effective muscle recovery following strenuous workouts.
Suggested Use: As a dietary supplement, add 3 heaping scoops to 8-12 fluid ounces of water. Mix and serve.

Form: 2.9 lb. Container; Chocolate. Vanilla and Orange Flavors
Serving Size: 3 heaping scoops (131 g)
Servings Per Container: 10

One Serving Provides: % U.S. RDA*
Calories 470 **
Carbohydrate (High Performance Branched-Chain Glucose Polymers and Pure Crystalline Fructose) 91 g **
Protein (Including di- and tri-peptides, free-form and branched chain amino acids, derived from whey protein hydrolysate) 24 g 53%
Fat 1 g **
Cholesterol 0 g **
Beta Carotene (Pro Vitamin A) 5000 IU 100%
Vitamin D 200 IU 50%
Pantothenic Acid 96 mg 960%
Vitamin C 60 mg 100%
Vitamin E 30 IU 100%
Niacin 20 mg 100%
Pyridoxine 2 mg 100%

Riboflavin 1.8 mg 106%
Thiamin 1.5 mg 100%
Folic Acid 400 mcg 100%
Biotin 300 mcg 100%
Vitamin B 126 mcg 100%
Magnesium 240 mg 60%
Manganese 2 mg **
Potassium 300 mg **
Zinc 3 mg 20%
Copper 1 mg 50%
Chromium 200 mcg **
Selenium 50 mcg **
Molybdenum 50 mcg **
Choline 100 mg **
Inositol 100 mg **
Trimethylglycine 75 mg **
L-Carnitine 50 mg **
* PAK (Pyridoxine Alpha-Ketoglutarate) 50 mg **
Lipoic Acid (Pyruvate Oxidation Co-Enzyme) 100 mcg **
PABA 10 mg **
Pantethine (Co-Enzyme A Precursor) 5 mg **

* Percent U.S. Recommended Daily Allowance for Adults.
**No U.S. RDA has been established.

Typical Amino Acid Profile:

L-Lysine 2369 mg
L-Histidine* 42 mg
L-Arginine** 720 mg
L-Aspartic Acid 2551 mg
L-Threonine* 104 mg
L-Serine 842 mg
L-Glutamic Acid 3876 mg
L-Proline 1145 mg
L-Alanine 1325 mg
Glycine 482 mg
L-Cysteine** 643 mg
L-Valine* 1366 mg
L-Methionine* 542 mg
L- Isoleucine* 1366 mg
L-Leucine* 2993 mg
L-Tyrosine** 12 mg
L-Phenylalanine* 965 mg
L-Tryptophan* 238 mg

* Essential amino acid.
** Conditionally essential amino acid.

Ingredients: Glucose Polymer, Whey Hydrolysate, Pure Crystalline Fructose, Natural Flavoring, Potassium Phosphate Dibasic, Magnesium Oxide, Choline Bitartrate, Calcium D-Pantothenate, Betaine Hydrochloride, Inositol, Ascorbic Acid, Inosine, L-Carnitine, Pyridoxine Alpha-Ketoglutarate, Copper Glycinate, Beta Carotene, Biotin, D-Alpha Tocopheryl Succinate, Niacinamide, Manganese Amino Acid Chelate, Zinc Picolinate, Citric Acid, PABA, L-Selenomethionine, Boron Citrate, Pantethine, Pyridoxine Hydrochloride, Riboflavin, Chromium Picolinate, Thiamin Mononitrate, Fumaric Acid, Succinic Acid, Vitamin B12, Folic Acid, Vitamin D3, L-Aspartic Acid, Malic

Acid, Lipoic Acid, Sodium Molybdate.

Item # PO

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PRO-OPTIMIZER

4 / case 2.9 lbs Powerful Anabolic Post-Workout Recovery Drink 24 Grams of Whey Protein Hydrolysate Per Serving High in Branched Chain Amino Acids

Pro-Optimizer contains high biological value amino acids from whey protein hydrolysate (enzymatically predigested lactalbumin), with ratio-balanced carbohydrates and a comprehensive micronutrient formula, providing building blocks and energy sources helpful for effective muscle recovery following strenuous workouts.

Serving Size: 3 heaping scoops (131g) Servings Per Container: 10

One Serving Provides: %U.S. RDA* Calories 470 ** Typical Amino Acid Profile: Carbohydrate (High Performance Branched-Chain Glucose Polymers and Pure Crystalline Fructose) 91g ** L-Lysine L-Histidine* L-Arginine** 2369mg 42mg 720mg Protein (including di- and tri-peptides, free-form and branched chain amino acids, derived from whey protein hydrolysate) 24g 53% L-Aspartic Acid L-Threonine* L-Serine L-Glutamic Acid 2551mg 104mg 842mg 3876mg Fat 1g ** L-Proline 1145mg Cholesterol 0g ** L-Alanine 1325mg Beta Carotene (Pro Vitamin A) 5000IU 100% Glycine 482mg Vitamin D 200IU 50% L-Cysteine** 643mg Pantothenic Acid 96mg 960% L-Valine* 1366mg Vitamin C 60mg 100% L-Methionine* 542mg Vitamin E 30IU 100* L-Isoleucine* 1366mg Niacin 20mg 100% L-Leucine* 2993mg Pyridoxine 2mg 100% L-Tyrosine** 12mg Riboflavin 1.8mg 106* L-Phenylalanine* 965mg Thiamin 1.5mg 100% L-Tryptophan* 238mg Folic Acid 400mcg 100* *Essential amino acids. **Conditionally essential amino acid. Biotin 300mcg 100% Vitamin B 125mcg 100% Magnesium 240mg 60% Potassium 300mg ** Zinc 3mg 20% Manganese 2mg ** Copper 1mg 50% Ingredients: Glucose Polymer, Whey Hydrolysate, Pure Crystalline Fructose, Natural Flavoring, Potassium Phosphate Dibasic, Magnesium Oxide, Choline Bitartrate, Calcium D-Pantothenate, Betaine Hydrochloride, Inositol, Ascorbic Acid, Inosine, L-Carnitine, Pyridoxine Alpha-Ketoglutarate, Copper Glycinate, Beta Carotene, Biotin, D-Alpha Tocopheryl Succinate, Niacinamide, Manganese Amino Acid Chelate, Zimc Picolinate, Citric Acid, PABA, L-Selenomethionine, Boron Citrate, Patethine, Pyridoxine Hydrochloride, Riboflavin, Chromium Picolinate, Thiamin Mononitrate, Fumaric Acid, Succinic Acid, Vitamin B12, Folic Acid, Vitamin D3, L-Aspartic Acid, Malic Acid, Lipoic Acid, Sodium Molybdate. Chromium 200mcg ** Selenium 50mcg ** Molybdenum 50mcg ** Choline 100mg ** Inositol 100mg ** Trimethylglycine 75mg ** L-Carnitine 50mg ** Inosine 43mg ** PAK (Pyridoxine Alpha-Ketoglutarate) 50mg ** Lipoic Acid (Pyruvate Oxidation Co-Enzyme) 100mcg ** PABA 10mg ** Pantethine (Co-Enzyme A Precursor) 5mg ** U.S. Recommended Daily Allowance for adults. ** No U.S. RDA has been established.

proop Regular price: $29.95 Sale price: **$25.46** flavors: chocolate

Order